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The Life History of the Fire Blight Pathogen, *Bacillus amylovorus*, as Related to the Means of Overwintering and Dissemination

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The disease commonly known as fire blight of pears, apples, quinces, and other plants is one that has been recognized in the United States for over 100 years. It has wiped out most of the commercial pear orchards in this country and is at times very destructive to apple orchards as well as to other fruit crops. In the history of agriculture the world over there are only a few cases on record which will compare in destructiveness to this disease. And in spite of the relatively large number of investigations that have been reported, the disease has rendered pear growing unprofitable over most of the country, and comparable in part to this, the disease is only being partially controlled on apples. In Arkansas the Jonathan apple as well as other susceptible varieties are often so badly blighted that yields are very materially reduced.

The specific objects of these investigations have been to determine, first, the relationship of different species of pomes to overwintering and dissemination of the disease producer; second, the relationship of different parts of the host to the harboring and over-wintering of the parasite.

For about 15 years apple growers of the Ozarks of Arkansas and of adjoining territory have firmly believed that pear trees are largely responsible for the fire blight disease on apples. This belief was based on the assumption that the causal agent, *Bacillus amylovorus*, lives over winter very largely in pears. It is therefore very pertinent to inquire into the evidence for any possible differential effect that unlike hosts of *Bacillus amylovorus* may have on overwintering and dissemination of the fire blight germ. If it is true that in the Ozarks the causal agent lives over mostly in pear trees and very little in apples, then it is quite obvious that the destruction of pear trees would help to exterminate the disease on apples to the great advantage of all apple growers. It should be noted that, in the region under discussion, commercial pear growing has become almost entirely a thing of the past. While numerous pear orchards formerly existed here, there are few now remaining, mainly because of the ravages of *B. amylovorus*, so that the pear trees which are left are for the most part located in home orchards, with a few small commercial orchards sparsely scattered over the counties. Two of these counties are now an important apple growing area.

The Literature on Disease Resistance and Susceptibility in Relation to Overwintering of the Pathogen

Out of the scores of articles that have been written on this disease, very few are concerned with any studies concerning the difference that may exist in unlike hosts with reference to the overwintering of the pathogen. Moreover, the little data that may be found partakes largely of the nature of field observations, with little or no effort to substantiate these with exact experimental methods. Many references are to be found to the resistance or susceptibility of different varieties and species of pomaceous plants to *B. amylovorus* and in numerous instances the list presented by one observer of susceptible and resistant varieties does not agree with that given by another. The following references contain lists of susceptible and resistant varieties: Crozier *(9)*, Hutt *(20)*, Whetzel *(49)*, D. H. Jones *(21)*, Sackett *(32)*, Hedrick and Howe *(16)*, Hewitt *(19)*, Stewart *(35)*, Cate *(7)*, Anderson *(1)*, Swingle *(37)*, Chambers *(8)*, Reimer *(29)*, Brooks *(5)*, and Supplements Nos. 1 (1919), 14 (1921), 20 (1922), 28 (1923), 33 (1924), 39 (1925), and 52 (1927) of the Plant Disease Reporter (Bulletin) issued by the Plant Disease Survey of the United States Department of Agriculture. The latter publications (mimeographed) present the most complete lists of resistant and susceptible varieties of apples to fire blight known to the writer. Of these, Supplement 33, compiled by C. R. Orton and J. I. Wood gives by far the most comprehensive lists on this subject. Moreover, with the exception to be discussed shortly, very little effort has been expended in correlating resistance with lack of ability to harbor the disease producer over winter. On *a priori* grounds it may be assumed that a species or variety of host that shows marked resistance cannot serve as a means of overwintering of the pathogen as readily as one that is very susceptible. But there are too many instances in human, animal, and plant pathology in which an immune or resistant individual acts as an important incubating agent for disease producing microorganisms. Thus among humans, epidemics of diphtheria, typhoid fever and other serious diseases are frequently traced to resistant carriers, while among plants there are various cases on record where a whole plant or certain of its parts were to all appearances perfectly normal and healthy and yet possessed infectious and disease producing agents. Among virus diseases this has been found to be true in a number of instances. It is quite obvious, therefore, that it is necessary to test carefully any susceptible or resistant variety of host before deciding as to its ability or inability to serve as a means of overwintering of any pathogen.

In order to clarify this discussion, attention is directed to the fact that two different phenomena are here involved. One

relates to the amount of blighting and quantity of inoculum produced during the periods of active growth by susceptible and resistant hosts, the other to the wintering-over of the pathogen in susceptible and resistant plants and its possible spread to healthy organs in the early part of the growing season. Obvi-

Fig. 1. Natural infections of fire blight on a species of Amelanchier cultivated for its fruit. (Photographed May 7, 1929.)

ously, a very susceptible plant is likely to produce a greater amount of inoculum during the growing season than a resistant one. Or, putting it another way, any medium conducive to the growth of *B. amylovorus* may be expected to yield greater numbers of this organism than a medium which does not readily

promote growth. But are we justified in assuming that because a particular medium does not promote a rapid growth, that the organism dies out more readily on such media? Numerous instances can be cited to the contrary.

What is the evidence for the assumption that *B. amylovorus* over-winters mostly on one group of susceptible plants, and, specifically, what evidence exists for the assumption that pear trees rather than apples are concerned with overwintering? The writer has gone through the literature on this disease very thoroughly and has been unable to find a single article in which an attempt has been made to prove that susceptibility to fire blight is correlated with the ability of the host to carry the pathogen over winter. It is true that numerous writers have called attention to the harmful influence that some susceptible variety of hawthorn, pear, apple, or quince has in acting as a source of inoculum for other species and varieties which are not so susceptible, but most of these references are concerned with the dissemination of the blight producer and not with differential overwintering. Certainly the investigators who have done most in studying this disease, including Burrill, Arthur, Waite, Whetzel, Stewart, D. H. Jones, Brooks and others, make no claim that pears are responsible for overwintering and not apples.

Very few investigators make definite statements concerning overwintering in only one species or varietal group. Hewitt in 1911 *(18)* and again in 1913 *(19)* took the position that "the blight organism lives over winter in only a few of the cases of limb blight on pear trees. The great majority of cases of blight, even on pear, die out before winter comes and the organism lives over winter only in the few hold-over cases. From these it spreads to the apple blossoms and twigs as well as to pear blossoms, the next spring, being carried mostly by insects. Blight never lives over winter on blossom clusters and very rarely on apple twigs" (*18* p. 426). No evidence is presented for these statements. In his later publication *(19)*, however, he gives the following evidence. "On the pear, fire blight sometimes grows very slowly downward but continues its slow growth through the winter, making what Waite called a *hold-over case*, because it is the only known means of holding the microbes over winter. The rapidly growing type of fire blight which causes greatest injury in the summer seems to be checked completely by the approach of cold weather. These holdover cases do not commonly occur on apples. In the present investigations, of more than a thousand trees examined, holdover cases were found only twice on apple trees active during April after having survived the winter. One of the cases was on Yellow Transparent, the other on McMahon". No information is given concerning the details of this work, whether it was based on

Fig. 2. Typical form of blossom and twig blight on Jonathan. Photographed May 13, 1926, when nearby pear trees showed no blight, while the tree bearing this limb was badly blighted.

field observations, attempts at culturing and isolating the disease producer, or whether twig infections were at all considered. Nothing is said of the number of pear cankers examined and

found to be "holdover cases" or in any case of any ooze observed prior to the first signs of blight.

D. B. Swingle in 1911 *(36)* and again in 1921 *(37)* constructed four groups of apple varieties relative to resistance and susceptibility and noted in the more resistant groups, named A and B, "the blight germs nearly always die out in the bark before winter and almost never live over until the next spring, while in those belonging to Class C (moderately susceptible, including among others Yellow Transparent and Delicious) they frequently live over winter, and in those of Class D (very susceptible) they quite regularly do so and thus start a new infection in the orchards at blossoming time". While Swingle may be correct in these assumptions, no evidence is presented to substantiate the contention that relative resistance in one group of hosts is correlated with lack of ability to carry the pathogen through the winter. It is, however, of considerable interest to note that, while Hewitt insisted that pear trees are primarily responsible for the overwintering of the bacteria, Swingle believed certain apple varieties are just as liable, if not more so, than pears, and says, "It is a misconception that pear trees growing in any locality are a special menace to the apple industry. Pears are no more likely to contract blight than apples; and if they have it and the owner desires to fight it he will find it not so difficult to combat in the pear as in the susceptible apples. The reason for this is that the normal bark of the pear is lighter in color and the blighted portions darker than in the apple; it is, therefore, easier to find all the blighted limbs." Of course, the discrepancy between Hewitt's conclusion and that of Swingle may in part be explained by the fact that while the former's observations involve Arkansas conditions the latter author dealt with conditions in Montana. This, however, does not explain the wide divergence between the two, for Swingle is fully aware of the marked susceptibility of certain pear varieties in Montana and calls attention to the fact that the Clapp's Favorite blights so badly that it cannot be grown commercially with profit. There are various other brief statements found in other publications in which it is assumed that overwintering occurs in one species and not in another, but, so far as the writer knows, no substantial evidence has been presented to confirm this view.

The Host Range of *B. amylovorus* in Arkansas

One of the first things to determine with reference to hosts in relationship to overwintering is the number and types of different plants to be found blighting in the region where these experiments were conducted. As a result of these studies several new and entirely unlooked for plants susceptible to blight were discovered. Among these were the Burbank plum and such common ornamental plants as the Vanhoutte spirea, the flowering

or Japanese quince, and cultivated roses. These results have already been published *(31)*.

Since the publication of the above article the writer has discovered two other hosts which appear to be entirely new to science. One is a species of Amelanchier (see Fig. 1) that is cultivated for its edible fruit, and the other is an oriental species

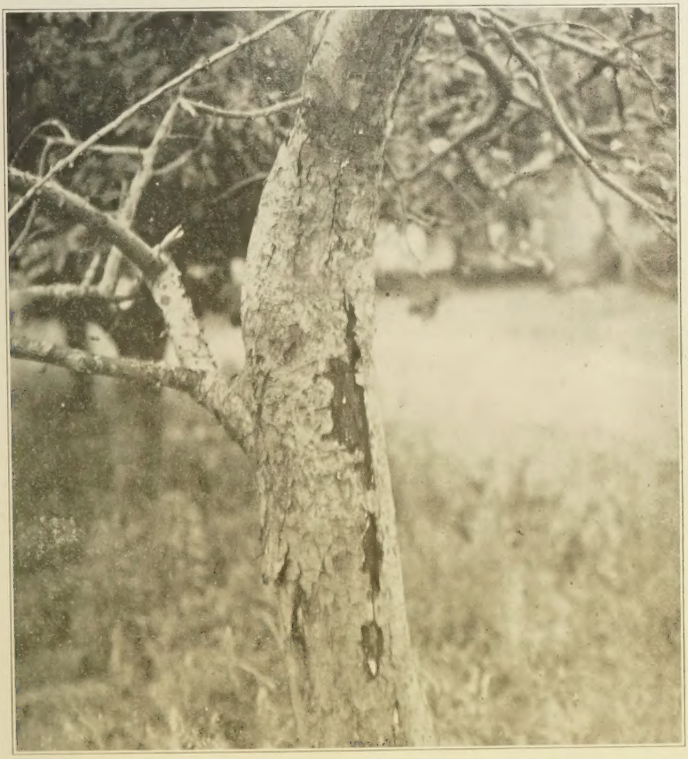

Fig. 3. Body canker on Jonathan apple photographed April 26, 1926. Fire blight killed this tree by the following season.

of Crataegus. The first of these was found infected in a mixed orchard of apple, pear and other fruit trees. The infections consisted of blighted blossom clusters involving symptoms that are similar to infections of apples and pears. From one of the blighted blossoms the organism was isolated and its pathogenicity determined by inoculating healthy pear shoots attached to

growing plants in the greenhouse and producing the typical blight symptoms. Compared to the number of Amelanchier blossom clusters which showed no signs of blight, the number of clusters found blighted were so few as to suggest that the disease is not particularly important on this host from the standpoint of damage done. On the oriental Crataegus the disease is far more serious, involving a blighting of twigs and even of large limbs in a relatively short time. Fortunately, this host, which might otherwise be considered an important ornamental and useful plant, is very rarely planted in this country. From this host the organism was also isolated and infections produced on healthy pear shoots.

Of the hosts that are commonly found blighted in Arkansas, pear and apple trees lead in the number and severity of infections. Strange to say, Crataegus species, despite their abundance, have not as yet been found blighted, although Reed *(27)* in Missouri reported blight on flower clusters of *C. crusgalli*, a species native to a large part of the country and presumably present in Arkansas. On the other hand, the fact that most of the blighting on Crataegus reported in the literature concerns the cultivated varieties of the English hawthorn, would suggest that native species are rarely infected. It is to be noted that, while native hawthorns are common in Arkansas, the introduced species are very scarce.

In addition to pear and apple trees, quinces are occasionally found with more or less blight, but in no case have they been found by the writer to be as severely infected as certain susceptible apple or pear varieties. As quinces are rarely grown in this state and as the blight hardly ever involves more than a few small twigs and blossom clusters, their importance as an overwintering agent or as a disseminating host during the growing season is negligible. In New York *(35)* they have been reported as very susceptible in the nursery row, but, as far as susceptibility of nursery stock is concerned, if quinces are to be excluded one might just as well exclude some of the most valuable commercial apple varieties grown in this state, such as the Jonathan, Yellow Transparent, Ada Red, Grimes Golden, and others, not to mention all common pear varieties, for these are all very susceptible to blight in the nursery. In this connection it is interesting to observe that Hesler and Whetzel (*17*, p. 386) consider the quince to rank below the pear and apple in susceptibility in orchards but in the nursery, "the order of susceptibility is as follows: quince, apple and pear".

Of the remaining hosts that have been found to blight under natural conditions in Arkansas, including the cultivated species of Amelanchier previously mentioned, the Burbank plum, reported in a previous article *(31)*, and the oriental Crataegus, the disease as a whole is relatively unimportant because of its scar-

city, or, as in the case of the last mentioned host, because it is rarely grown. Cultivated crabapples, such as the Transcedent and Whitney crabs, which have been noted by numerous investigators as extremely susceptible in the more northern parts of the United States, are seldom grown in Arkansas. Blight has been found by the writer on the few trees that have come under his observation, but the scarcity of the hosts would, in this case also, exclude them as important disseminating agents. Of the trees that have been noted, blight was

Fig. 4. Upper row: First spring infections on apple, Jonathan variety; lower row: healthy fruit clusters for comparison. (Photographed April 23, 1929.)

not as serious as in certain nearby varieties of apples or of pears, but this may have been due to some factor not particularly connected with the inherent qualities of the trees but to some fortuitous or external condition, such as lack of bloom or comparative freedom of inoculum when the trees were in a susceptible stage. Wild crabapple trees are known to be present in the state, but no blight has as yet been observed on them. Likewise, the apricot and other stone fruits, the mountain ash, and other hosts which are known to be susceptible in other parts of the country, have not been found blighted in Arkansas.

General Field Studies of Resistance and Susceptibility of Apples and Pears in Relation to the Overwintering of the Pathogen

Because by far the most important hosts of this disease in Arkansas have been found to be apple and pear varieties, the investigations which have thus far been conducted had to be limited to these. On these hosts the investigations as already mentioned concerned the relationship of different varieties of apples and pears to overwintering and dissemination of the pathogen, and the relationship of different parts of the host to overwintering.

In order to determine the facts, a large amount of field observations coupled with microscopic examination of diseased tissues, including histological sectioning of diseased wood, attempts at culturing and isolating the parasite, and of artificial inoculations into susceptible plants growing in the greenhouse, were attempted.

Of the field observations, attention was at first centered on an orchard of several acres of apple trees, approximately one acre of pear trees and several more or less localized groups of apple and pear trees growing around Fayetteville. All the trees chosen for intensive observation were within a few minutes walk from the laboratory. Later the field observations were broadened so as to include numerous commercial orchards as well as home or back-yard apple and pear trees in diverse localities of Washington and Benton Counties. The number of trees that have been under observation has not been definitely recorded but would easily involve several thousand. In these observations the writer was assisted at times by A. B. Groves and Luther Shaw. Particular attention was paid to certain susceptible varieties of apples including the Esopus Spitzenberg, Yellow Transparent, Ada Red, Jonathan, (Figs. 2 and 3), Maiden Blush, and Grimes Golden. Of these, the first variety was represented by only three trees, whereas the others included several large sized commercial orchards as well as trees in home orchards. Very little attention was paid to those apple varieties which show resistance to blight in Arkansas. Thus Ben Davis, Black Bens, Staymen Winesap, Winesap, and Delicious were almost wholly excluded from these studies.

Of the pear varieties under observation, the Kieffer was by far the most common, but in addition to this the following varieties located in the varietal test orchard of the University horticultural department were also intensively studied: Lincolin (6 trees), Duchess (3 trees), Anjou (2 trees), Garber (4 trees), Seckel (2 trees), Bartlett (3 trees), Sheldon (3 trees), Comice (2 trees), Winter Nelis (4 trees), Flemish Beauty (2 trees). Most of these were 5 to 8 years old when the studies were begun

in 1926, and, with the exception of the Garber and Seckel, the other varieties bloomed more or less profusely in one or more years since that time.

The field observations consisted, first, of ascertaining the relative susceptibility of different varieties of apples and pears; second, a close inspection of all cankers, large and small, during various parts of the year and especially in the early spring, on certain badly diseased apple and pear trees, for signs of pathogenic activities, including the production of ooze; and, third, the possible influence of any one host on another in the dissemination of the disease producer and the production of disease.

Fig. 5. First spring infection on pear, variety Winter Nelis. Note the evidence for infection through the flowers in the dark, discolored appearance of the floral organs and upper pedicels when the lower portions of pedicels are still healthy; also note one young fruit without evidence of disease. (Photographed April 16, 1929.)

In determining the susceptibility of different varieties of apple and pear trees, a number of factors were taken into consideration, particularly the following: first, the previous treatment of the trees under observation, including pruning, the kind and amount of fertilizer, and cultural practices; second, the general vigor or growing condition; and, third, the amount of bloom. In view of the fact that any one of these factors, as well as others not mentioned, may materially influence the amount and severity of the disease on any one tree or group of trees, it is obvious that unless they are taken into consideration errors

are likely to be made in ascribing resistance or susceptibility to different varieties and species. It is mainly due to this that so many discrepancies occur in the literature concerning varietal resistance and susceptibility. As a whole, however, pathologists, as well as the more progressive type of fruit grower, have recognized the influence of these factors, although the relation of bloom to blight development has not been recognized as fully as it should be. While twig blight as well as limb and body blight may frequently be found in the absence of blossom blight, it may be noted that as far as concerns Arkansas orchards blight of any kind is likely to be present only in minor proportions in the absence of bloom blight. In other words, where there is little bloom development, or when some weather or growing condition injures the bloom during its development, then blight is seldom serious on such trees. During the three years in which this disease has been intensively investigated and in the opportunities that have been afforded for seven years previously to note the occurrence of diverse diseases including fire blight, very few cases have been observed in which a tree severely infected with twig and limb blight had not first suffered from blossom blight (Figs. 4 and 5). Two such exceptional cases which have been noted consisted of a severe twig blighting on Maiden Blush trees which had produced very little bloom. The trees were growing in a mixed orchard of various fruit trees, including several apple varieties and one Kieffer pear tree, on ground which had been heavily fertilized with manure the previous year. Under such conditions blight, if the pathogen is at all present, is apt to be very serious, as has been reported by numerous observers. It has been well known, particularly since Stewart's *(35)* work in 1913 on blight in nursery stock, that blight may be severe in the absence of blossom blight, but this as a whole is limited to the nursery row, to young orchard trees and to older ones in which susceptibility has been enhanced by some manurial, cultural, or pruning treatment.

With these criteria in mind it may be stated that field observations indicate that as a whole blight is apt to be more severe on the varieties of pears grown in Arkansas than on the susceptible varieties of apples which are commonly grown in this state (see varietal lists of apples and pears given previously). The Kieffer and to a less extent the Garber pears are by far the most common varieties to be found. While these are considered by pathologists in northern and western states as more resistant to blight than such varieties as Bartlett and Clapp's Favorite, nevertheless in Arkansas, as well as in other states of the south, it is safe to say that the Kieffer and Garber are very susceptible. This does not mean that they always blight or that they are as susceptible as the Bartlett, but that under

southern conditions they are apt to show more blight than the susceptible varieties of apples grown in this region. However, as will be shown later, this is by no means the case at all times or under all conditions. Moreover, the Lincoln pear has borne heavily and has shown very little blight when nearby Kieffer's and apple varieties such as Yellow Transparent, Maiden Blush, Jonathan, and Esopus Spitzenberg were badly diseased, thus suggesting that even among pears there may be varieties which are not as susceptible to blight as are some apple varieties. Anderson *(1)* in Illinois has also noted the comparative resistance of the Lincoln pear. On the other hand, Archer *(2)* found this variety severely infected in Iowa in scion orchards and only slightly so in an experimental orchard.

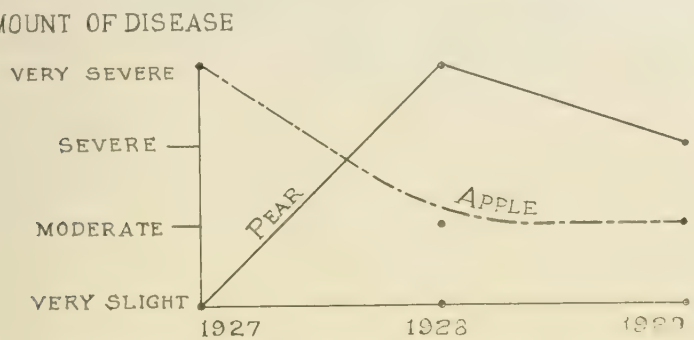

Fig. 6. Graphic summary of blight on pears and apples during 1927, 1928 and 1929.

By the statement that blight is apt to be more severe on pears than on apples is meant that a badly infected pear tree will usually show more killed blossom and fruit clusters or more dead and dying twig and limb tissues than is usual in a severely infected apple tree. There are several possible explanations why this is particularly true in the Ozarks of Arkansas. These are: (1) earliness of bloom of Kieffer which is possibly coupled with a timeliness of inoculum dissemination and especially of the degree of susceptibility of pear blooms; (2) rapidity of early-season growth; (3) succulence and thickness of bark; and (4) lack of proper care of pears compared to apples, especially the neglect to prune out diseased wood. Aside from any inherent chemical or physical factors present within the cells, which may be associated with susceptibility or resistance, there are a number of conditions, environmental—natural or man-made—which materially influence infection and disease production, often resulting in a differential action on unlike hosts.

In order to understand more clearly the reasons for the

greater amount of blight encountered on pears and also to determine the possible relationship of early pear infections to subsequent epidemics of apple blight, a number of field studies were undertaken. During the three seasons in which this disease has been intensively studied, a careful record has been kept of daily observations throughout the late dormant and growing season, including the months of February, March, April and May. These included, among other things, any possible oozing from overwintered cankers, the flowering period of various apple and pear varieties, weather conditions especially at blooming periods, observations on the date of first signs of blight and its possible relationship to any nearby infectious material, and the severity of blight on different hosts. Coupled with these field studies, twigs, limbs and tissues from body cankers, all representing blighted material from the previous season, were taken into the laboratory throughout the year, particularly during the winter and early spring, for careful microscopic studies and for attempts at culturing the pathogen.

Table 1 presents in brief the field observations for 1927, 1928 and 1929 on two varieties that are commonly grown in this state, one of pears and the other of apples, both being very susceptible to blight. It will be seen that the Kieffer pear is usually past the blooming period when the Jonathan apple is beginning to bloom. Hence, it would not be surprising to find blossom infections on Kieffer prior to those on Jonathan, unless something happened to the Kieffer blooms, such as frost injury or other adverse conditions, or the absence of inoculum at the time of blooming. It would burden the record to give all the data that have been gathered on the blooming periods of various apple and pear varieties, but the results may be summarized in the statement that, of all the pear and apple varieties that are commonly grown in Arkansas, the former bloom one to three weeks earlier than most apple varieties. The Duchess apple, one that

TABLE 1.

	Host	Flowering period	Frost injury to blooms	Date of observation of first signs of blight	Relative prevalence of blight during season
1927	Kieffer	March 7 to 18	95% killed	April 16	Very slight
	Jonathan	March 17 to April 5	10% killed	April 15	Very severe
1928	Kieffer	March 17 to April 3	50% killed	April 23	Very severe
	Jonathan	April 3 to May 1	20% killed	April 29	Moderate to severe
1929	Kieffer	March 17 to April 3	Absent	April 10	Severe
	Jonathan	March 29 to April 15	Absent	April 16	Moderate

is not very commonly grown in Arkansas, blooms before Jonathan and may be found in full bloom within a few days after the main crop of Kieffer blooms have opened. (In the table the blooming period is considered to be the time intervening between the separating of the individual flower buds within the cluster and the shedding of most of the petals.) It is quite obvious, therefore, that if blossom blight occurs it may be expected to appear first on the pears. Granting this and also that twig blight, which in Arkansas as a whole may be traced directly or indirectly to blossom blight, is apt to be found first on pears, is this evidence for the assumption that the pear is mostly responsible for harboring the germ over winter? Then again, assuming that the prior development of blossom blight on pears increases the sources as well as the quantities of inoculum available at the blooming time of apples, does this offer any evidence as to the relative amount of overwintering on the two hosts?

While field observations clearly indicate that susceptible varieties of apples growing near blighted pear trees are apt to suffer more from blight than apples otherwise situated, nevertheless the studies clearly show that apple blight can be very serious either in the absence of nearby pear trees or when pear trees which are not infected are present. Thus an inspection of Table 1 shows that in 1927 there was a very severe epidemic of apple blight in Arkansas when there was but a slight amount of pear blight, and this in turn may be partly explained by noting that the early frosts had killed 95 per cent of the pear blossoms. Conversely, it is to be noted that in 1928 and in 1929, when pear blight was very serious, there was only a moderate amount of apple blight. This, of course, does not mean that all pear or apple trees of a given variety behaved alike with reference to blight during any one season but that, in averaging the blight noted in various localities, no general relationship was discovered between the amount of pear blight and of blight on apples (see graphic summary p. 15). Indeed, in 1927 and in 1928 a number of localized cases were noted in which blight appeared first on apples and was not observed on pears until later in the growing season. Thus in 1927, when the first blight was detected on a Jonathan apple on April 15, an orchard of 31 pear trees (varietal test orchard previously mentioned), which was not more than 50 yards away from the infected Jonathans, showed no signs of blight until April 26, 11 days later.

Among the possible factors which have previously been enumerated as contributing to the greater amount of blight on pears in Arkansas was negligence in pruning. It is very common to see apple orchards in the Ozarks of Arkansas and Missouri receive careful attention in the way of pruning and spraying while pears are frequently neglected. The chief reason for this is that pears are rarely grown for commercial purposes and,

coupled with the fact that they are apt to have more blighted wood, makes pruning a relatively expensive and time consuming operation on pears. It is by no means true that all apple trees receive careful attention while pears are always neglected. While the commercial apple grower must perforce use great care in his apple orchard if he is to profit on his investment, this does not indicate that he will take equal care of his few pear trees nor does it indicate that those who are not in the commercial apple business will use any more precaution in removing blighted material on apples than on pears. From this point of view, it would be just as reasonable for an apple grower to request his neighbor to remove the neglected apple trees as it is for him to request the removal of pears.

Observations on Oozing

If there is any part of the field studies that has received closer attention than any other, it is that which pertains to the activities of blighted parts of pear and apple trees in the late winter and early spring, prior to and during the time of blooming. The specific object was to note as many cases of oozing as possible. It may be stated that the prevailing opinion concerning overwintering of the parasite, and the manner in which infections are brought about in the early spring, is as follows: "In certain cases the germs manage to keep alive through the summer by making slow progress in the fleshy bark. Such cases may succeed in living over winter. . . . The cases of 'hold-over' blight start off again in spring and exude quantities of gummy matter full of the bacilli. This is visited by insects, especially flies and wasps, and carried on to the newly-opened flowers. . . ." Waite *(45)*. This view together with Waite's *(40-42)* earlier observations on the relationship of bees to blossom infections have been almost universally accepted as established facts by pathologists and fruit growers. Up until very recently the only modification that has been made since 1898 is the discovery by Sackett *(33)*, Stewart *(35)*, Brooks *(5)* and Tullis *(38)* that very small blighted twigs as well as large limb and body cankers are capable of carrying the pathogen through the winter.

It is necessary to point out,however, that the evidence tracing the first spring infections to the oozing of hold-over cankers and to the dissemination of this bacterial ooze by insects has not yet been presented *(40-48)*, and the very recent work of Tullis *(38)* and of Miller *(23)* would tend to question the role of insects in spreading the blight producer to blossoms in the early spring.

Several other investigators who have made special studies of this disease have concerned themselves more or less with the problem of overwintering of the pathogen. Among these are D. H. Jones *(21)*, Gossard and Walton *(13)*, Brooks *(5)*, and

Miller and Keitt (24). Of these, Jones, and Gossard and Walton were either unable to detect any oozing prior to the first signs of blight or noted such few cases that they questioned the efficacy of this in initiating blight. Brooks, and Miller and Keitt present contrary evidence. Jones very evidently made a determined effort to detect such oozing (p. 23). "The question is, where do the bees in the first place get the contamination? As previously remarked, when the disease is in an active condition either in twig, bark or fruit, a gummy exudate loaded with germs is often found oozing through the epidermis of the affected part. Insects alighting upon or feeding on this material would get contaminated with the germ and carry it away with them attached to their feet and mouth parts particularly. We have found flies, beetles, aphids and other hemiptera feeding on or walking over this area. Though possibly this is how the bee gets contaminated in the first place, the writer has never yet seen one alight on the gummy exudate, and in fact though careful search was made last season through the College orchard and all through blossom time, no gummy exudate could be found on twig, branch or trunk of apple or pear tree. Later in the season, however, in the latter part of June, throughout July and August, when the disease was active, much exudate was observed on trunk, twig and branch of affected trees, and many trees were affected. Notwithtanding the fact that no exudate was observed on the trees before or during blossom time, a large number of blossom infections occurred and subsequently developed in various parts of the orchard. If these inoculations were made by bees from where did the bees get the germ, if there was no gummy exudate for them to come in contact with?"

Fig. 7. Two-year old apple twig with a blight canker. Material gathered on March 4 and kept standing with base in water in the laboratory until March 23, when a droplet of ooze was noted on the old blighted part. This ooze proved to be non-infectious. (Photographed March 23, 1928.)

Gossard and Walton, likewise, attempted to find oozing before

Fig. 8. Spitzenberg apple twig with the margin of the previous year's canker marked (near middle), gathered on April 4, 1928, and kept with its base immersed in water in the laboratory for about one month. By this time the margin of the canker extended so as to include the base of the shoot, seen to the right. On April 24 a small droplet of ooze was noted at the margin of last year's blight and this proved to be infectious.

blight development and their statements on this are interesting (p. 117). "After watching closely for several seasons at Wooster, we affirm that very few cankers in this locality have become active by blossoming time, and that before the bloom only a few accidental insect visitors come in contact with them at all. In 1916 we could only locate three active cankers before blooming and we saw no insects at all visiting them. One canker

was exuding April 7 and judging by the exudate must have become active by April 1. Another was found shortly after April 7, and the third April 25. In 1915 we knew of three cankers slightly active before blooming, and by watching for several hours per day on several different days saw two or three ants, an elaterid, and a lampyrid or two crossing over them in a perfectly normal manner, not stopping to feed nor giving the slightest hint that they were in any way interested in the area over which they traveled. We felt justified in doubting whether these cankers had any connection with the wave of blossom blight which a little later swept over the entire orchard." Gossard and Walton believe that spring infection comes from rain washing over these exuding cankers and then dashing over open blossoms and twigs. But they present no evidence for this belief and are evidently skeptical about it as may be seen by the following statement (p. 118). "However, when a whole orchard of 40 to 60 acres exhibits blossom blight appearing suddenly over its whole extent, when only two or three or no hold-over cankers at all could be found previous to the outbreak, one naturally concludes that the infection was carried into the orchard from an outside source, and that very little of it can justly be ascribed to resident hold-over cankers." This led them to postulate "that a wave of blossom blight travels northward with the bloom" being initiated in some southern state and accentuated by the few hold-over cankers to be met on its way northward.

Fig. 9. Blighted Jonathan apple twig, collected Dec. 16, 1926, and kept in laboratory until Feb. 21, with its base immersed in water, showing an indefinite margin which had extended downward for about four mm. after it had been collected, but isolations from this were negative.

Brooks' studies on epidemics of fire blight in Wisconsin (5) involve considerable work on early spring infections in relationship to the overwintering of the pathogen. Unlike Jones, and Gossard and Walton, he seems to have had no difficulty in finding oozing hold-over cankers prior to the first signs of blight, and the production of ooze or exudate plus the extension of blighted areas were used by him as criteria in showing that

a number of apple varieties carried the disease producer through the winter. Not only did he find exudate on large limb or body cankers but also on overwintered twigs. Thus, out of about 12,000 blighted apple twigs, he noted typical bacterial exudation in 2.5 per cent of such twigs of McMahon, 0.6 per cent in Fameuse, and 1.5 per cent in Wealthy. However, he presents no data to show that such twigs served as the source of inoculum for the first blight, although he does attempt to show such relationship in connection with some other studies. These involve records gathered in one orchard at Gays Mills, Wisconsin, in 1925. In this orchard the first blight was found on shoots and not on blossoms and "upon close observation it was found that these infected shoots formed cone-shaped areas in the trees, and at the apices of these cones were found actively exuding holdover cankers or twigs." But when his actual data, shown in his graphic summary, Fig. 6, p. 678, is examined it is to be seriously questioned whether proof is given for this contention. While the period of incubation, revealed by artificial inoculations, was found to be about 13 days, the length of time intervening between the detection of the first signs of oozing and that of blighting is only 5 days. If Brooks' dates and figures are accurate and if he overlooked no oozing on dates previous to those noted, then, from his own data one is forced to conclude either that the period of incubation of his artificial infections does not indicate the incubation period of natural infections or that the oozing noted had no connection with the first blight development. Of these two hypotheses the first is less apt to be true because in artificial inoculations the period of incubation is apt to be shorter than in natural inoculations. The use of puncturing instruments and relatively large quantities of pure culture inoculum, the latter being often used in such work, make conditions for artificial infections more ideal than is likely to occur under natural conditions. (Artificial infections on blossoms were not attempted.) But, whatever the explanation may be for this discrepancy, it is evident that Brooks has not proved that the first spring infections are due to the oozing of overwintered cankers or twigs. It should be noted, however, that, while one may question his data relative to the initiation of blight by means of oozing, overwintered cankers, his discovery of cones of infection and his thoroughgoing studies of overwintering in blighted twigs have been very valuable.

Coming to the recent work of Miller and Keitt (24), which is in the nature of a preliminary report presumably to be followed by a more adequate exposition, there is now presented more definite data concerning the activity of overwintered cankers and their initiation of blight. They noted bacterial oozing of cankers in 1927 at Gay's Mills, Wisconsin, on April 19, about three weeks before the beginning of the blooming period. "The

first blighted flower bud clusters and twigs of the season were found before the blossoms opened. In each observed case of this early infection, the blighted flower buds or twigs were located just below an active canker or blighted twig in a position favorable for infection by water-borne inoculum. No evidence of an insect-borne primary inoculum was observed." The date of the appearance of the first blight is not given, but the inference is that oozing occurred early enough to account for the appearance of the blight immediately below the active cankers and that the inoculum was carried by rain washings rather than by insects.

Fig. 10. Apple twigs from which infectious material was obtained during the winter of 1928 (see Table 2). Starting with the one on the left, the dates in which these were gathered are Dec. 9, 14, and 20, and Jan. 6.

This latter theory is in accord with another brief paper by Miller *(23)* and with the more substantial publication by Tullis *(38)*. While it cannot be doubted that Miller and Keitt have presented the best evidence up to date for the initiation of blight from overwintered cankers, it is seen that even in their publication the evidence is far from being complete.[1] Numerous other accounts are to be found in various publications on the activities of overwintered cankers and the production of spring blight, but

[1]While this bulletin was being printed, Miller's article entitled "Studies of Fire Blight of Apple in Wisconsin" appeared (Jour. Agr. Res. 39: 579-621, illus., 1929). In this publication evidence is presented to substantiate Miller and Keitt's and Miller's previous statements. No adequate analysis of Miller's data is possible at this time but it may be noted that proof of overwintering and early spring dissemination from exuding cankers is offered in the form of graphs, which among other things include the dates of early spring oozing and of first spring blight. If the writer is correctly interpreting Miller's data, Miller found three exuding cankers active before the first signs of fresh blight in 1926, 2 cankers in 1927 and one in 1928. Such numbers are very comparable to those found by Gossard and Walton *(13)*.

in no case, as far as the writer knows, has adequate evidence been presented to substantiate this hypothesis.

With this exposition of the previous work on early spring oozing in relation to the first blight, the data gathered in Arkansas on this subject is presented. Attention is called first to the very excellent facilities afforded for studying this problem because of the nearness of blighted pear and apple trees to the laboratory, making possible an intensive daily, field study as well as affording the use of refined laboratory equipment. Thus, if at any time some microscopic study or pure culture isolation indicated something contrary to the field observations, it was possible to return to the field with very little loss of time to obtain additional data. The most surprising observations to be recorded of the present studies is that for the years 1927, 1928, and 1929 no oozing of disease producing bacteria was observed prior to the first signs of blight. In spite of the most intensive sort of study of blighted material, involving in certain instances the daily use of a ladder to make sure that every possible bit of blighted material scattered over the trees was under close scrutiny, the failure to detect bacterial oozing prior to blight development is complete. Attention was especially focused on certain pear and apple trees that had developed considerable blight in the season previous to the observations and in which the blighted parts had not been pruned out. One ladder was kept specifically for this purpose and if any portion of a tree could not be carefully observed it was severed. It can be said with reasonable certainty that of several acres of apple trees and of about one acre of pear trees, that no oozing of *B. amylororus* above ground occurred prior to the first signs of blight. All of these were close to the laboratory and in some of them blight was at times extremely common and serious, involving body cankers, limb cankers, as well as many blossom and twig infections. As the sap begins to rise in the trees in early spring, there is likely to be a flow of gummy material from injured parts. In a number of instances this gummy material was noted in or at the margin of various cankers and blighted twigs. (See Fig. 7.) Such ooze or gum was always subjected, first, to microscopic observations, second, to attempts at isolating the pathogen, and, third, to inoculations into very young, vigorous Bartlett pear shoots, attached to trees maintained in the greenhouse. In the latter tests, checks involving inoculations with pure cultures, were always conducted simultaneously with the inoculations from the suspected materials. The records show that, while the checks never failed to show successful infections in one or more twigs, the gummy ooze has yet to yield such infections. Microscopic observations of this material has invariably shown the presence of bacteria, sometimes mixed in with various types of pollen grains and fungus spores, and the plate cultures at times yielded

Fig. 11. Two Jonathan apple twigs gathered May 3, 1928, with blighted shoots, one at the left with no ooze in the subtending twig canker and the one at right with a drop of ooze at upper margin of last year's canker.

colonies which suggested *B. amylovorus*. But inoculations with pure cultures of such colonies, as well as the direct inoculations with the gum itself, slightly diluted with sterile water, have failed to produce infections.

In order to study carefully the daily behavior of individual cankers and twig infections throughout the early spring, some 1,200 cankers mostly on Jonathan apple were tagged on the

trees and the margin outlined with water-proof ink and with wax pencils. Two Jonathan trees which had suffered very severely from blight in 1926 and which had received no pruning were made the special objects of study in 1927. The cursory field observations of the former year had indicated that these trees occupied the center of an infection area which involved a large number of nearby pear and apple trees. Because of the severity of blight on these Jonathan trees, plus the fact that they seem to have been utterly neglected in former years while the nearby trees received more attention, made them fit objects for careful study of overwintering. Also in 1926 a third Jonathan, which had stood within 25 feet from the other two, had suffered so severely from blight, including a large body canker, (see Fig. 3), that it failed to send out a single shoot the next spring, having died completely by the spring of 1927. For convenience in keeping records, the cankers and blighted twigs of each tree were labeled as follows: top of tree cankers and blighted twigs 1 to 100, east side 101 to 200, west side 201 to 300, north side 301 to 400, south side 401 to 500. All the remaining blighted wood was removed and all markings of margin of cankers and of diseased areas of blighted twigs, plus the placing of tags, was done in the middle of February. In this manner daily records of 500 blighted twigs and cankers were kept of each tree. In the same manner the records of about 200 additional cankers were kept of several Kieffer pear trees and of a Spitzenberg apple. Similar studies were made for three successive years, the number of trees and the areas of observation being enlarged so as to include about five acres of apples and about 10 acres of pears, the latter being mostly located near Bentonville.

The eight-acre block of 25-year old Kieffer pear trees near Bentonville offered exceptionally favorable opportunities for studying the field behavior of blight on pears, since a relatively large amount of blighted wood, including body and limb cankers, was present. The amount of blighted material was so great that it was practically impossible to go over all the trees as carefully and as completely as was done for the Jonathan apples. In 1927 and 1928 time permitted only a few inspection trips to this orchard, the inspections occurring in late February, March and April. As these were considered insufficient, it was decided to make as many inspections as possible in 1929. Beginning with February 28 these were conducted once a week up until the middle of May, when several weeks had intervened since the blight was first observed in this orchard. The remaining pear trees, about 50 in number, were all centered around the blighted Jonathans and were as carefully observed as the latter. Of these 50 trees, 31 had received careful pruning up until 1927, but from then on pruning was intentionally neglected so as to permit a study of as much blighted material as possible. Notwithstanding

Fig. 12. Two Spitzenberg twigs with new blight developed near the margin of last year's blight, gathered May 17, 1928. Bacteria were observed in the ducts between the old and the new infections. Is this evidence for the internal extension of blight, since no external oozing of the old cankers was noted? (See Fig. 13.)

the care and the large amount of labor devoted to this work, all the observations on oozing were negative.

The single case of oozing observed from a hold-over canker occurred in the laboratory. In the course of histological and cultural studies of blighted wood, which is to be detailed later,

numerous severed twigs and branches were brought into the laboratory, and if the material was not to be studied immediately the cut ends were usually immersed in a vessel of water. One of the limbs thus handled was severed on April 4, 1928, from a Spitzenberg apple tree which showed a relatively large amount of blighted twigs and limbs. It was a three-year old limb, 1.25 inches in diameter, and the blight of the previous season appeared as an indefinite blackish, slightly sunken canker (the term "canker" is used variously) with the lenticels much more prominent than is usual in healthy limbs of this variety. No corky layer or crack differentiated the diseased from the healthy tissues. The margin of the discolored area was outlined with a wax pencil on the day the limb was gathered. The same sort of procedure was followed at different times in about 500 other cases representing a diverse assortment of cankers and blighted twigs from various apple and pear varieties. The tree bearing the limb under discussion was beginning to bud at the time, and some of the leafy buds were slightly open. The twig with its cut end immersed in water remained in the laboratory for over one month during which time two leafy shoots developed to a height of about 2.5 inches. In the meantime the margin of the canker had extended downward for about five inches at the widest point of extension (see Fig. 8), and one of the leafy shoots which had developed at the apex of a small twig, which in turn was attached to the cankered limb, gradually withered, while the subtending twig with its base now involved within the extending canker showed distinct signs of blight. The bark became darkened, took on a more or less metallic, water-soaked appearance and appeared blistered. On April 24 a small droplet of ooze was noted at the margin of last year's blight, and when part of this was mounted in water and examined under the microscope it was seen to be made up almost entirely of bacteria. The remainder, handled with sterile forceps, was macerated in sterile water and inoculated with a hypodermic needle into a young Bartlett pear shoot growing in the greenhouse. Twelve days after inoculation this shoot showed distinct signs of blight. From it the organism was isolated in pure culture and artificial infections were obtained on Bartlett pear shoots, with the production of typical ooze within three days after inoculation. (It may be of interest to note that with pure culture inoculum applied as a hypodermic injection the period of incubation in young, vigorous, Bartlett pear shoots can be reduced to as low as 24 hours, under proper greenhouse conditions.) The evidence is, therefore, substantial that the droplet of ooze obtained from this canker contained virulent bacteria. It is to be emphasized that this adhesive, very sticky, droplet was not more than about one-eighth of an inch in diameter. What is the significance of this drop of ooze? Is it due to abnormal, laboratory conditions and of

no particular relation to field behavior, or may it be regarded as a phenomenon that is likely to occur in the field? What is the possibility that in the field the first oozing may be so scarce and in the form of such small droplets as to defy detection? The writer has no definite answers to these questions as yet, but work along this line will be continued. In the meantime, the findings up to the present leave no doubt that a great deal of the prevailing opinions concerning the significance of oozing

Fig. 13. Cross section of a Spitzenberg apple twig gathered May 26, 1928, showing bacteria in ducts, section taken from tissue immediately below the margin of newly blighted wood. About three inches farther down appeared a canker initiated the previous season and the discolored xylem joined the old and the new blight. Is the new blight merely an extension of the old? Magnified about 500 times. (See Fig. 12.)

for initiation of spring blight is badly in need of confirmation. It is to be noted that in 1928 the first signs of blight on apples were noticed on April 29, five days later than the detection of the oozing in the apple limb kept in the laboratory. Such an interval of time is entirely insufficient to cover the incubation period necessary for the disease to appear after the bacteria have been applied to susceptible tissues. It will be shown later that the incubation period in the early spring under out-of-door conditions is from 10 to 14 days. Furthermore, even if one considers a five-day interval as a sufficient incubation period under

certain unknown conditions, it remains to be shown that the oozing observed in the laboratory under the peculiarly forced conditions of even indoor temperature and artificial watering has any relationship to out-of-door phenomena.

The many statements to be found in the literature relative to active or exuding cankers prior to blight development, leaving the very evident inference that the prevalence of the ooze and size of the droplets make oozing readily observable, can be put down as having no data for their substantiation and that the oozing referred to may have occurred during or after the first blight had developed. Once a flower cluster, leafy shoot, twig or limb is freshly infected, it is often to be found oozing copiously. From his observations in the years 1927, 1928 and 1929, the writer is ready to question all previous statements concerning early oozing and its role in starting blight that have been made for the region within the Ozarks of Arkansas and Missouri; specifically, he is ready to reject completely the assumption that such oozing is common on any hosts and that it is readily observable. However, he is not prepared to do so for other localities within the United States. Conceivably the behavior of cankers relative to oozing may be quite different on unlike varieties and in diverse parts of the country. The great importance of this question necessitates a complete review of the sources of spring inoculum, the relationship of bees and other insects to first infections, and the question of rain and wind as early disseminating agents.

In addition to observations of any possible oozing, close attention in this study was also paid to any other possible signs of activity of overwintered cankers and blighted twigs. As already mentioned, some 1,200 cankers in 1927 were outlined in the late winter and observed daily thereafter for indications of extension into healthy wood. The first signs of extension were noted on March 4, involving four different blighted twigs, the new discolorations extending for about 1 to 4 mm. beyond the old blighted wood. From two of these, isolations were attempted by using the wood found on both sides of the old margins, including the portions involved in the recent extensions. In one case the twig was carefully cleaned in running water, the outer bark removed with flamed knives, and the remaining tissue ground in a sterilized mortar. This was mixed with nutrient dextrose broth and used for a series of poured plates. In the other case the same procedure was used, except that the tissue was macerated in a petri dish containing nutrient dextrose broth. In both cases the bacterial colonies isolated, which by their color, size and shape suggested *B. amylovorus*, failed to produce infections on young, vigorous pear shoots. On March 5 three other small extensions were noted into healthy tissues, one on a three-year old limb, another on a two-year old twig,

Fig. 14. Hold-over blight on three Jonathan limbs which produced cone-shaped areas of infection. Note particularly the constrictions on the limbs at the upper right and left with the fresh blight extending into the thickest parts above the constrictions. Note also the fungus fruiting bodies on the old infected tissues in the left-hand limb in contrast to the newly infected extensions which are devoid of such fruiting bodies. Gathered July 19, 1929 near Farmington, Arkansas. About natural size.

and the third on a one-year old twig. The isolations attempted from the two-year-old twig failed to produce infections. Up to April 15, when the first blight was noted, no other extensions were observed. In the two succeeding years similar studies were made and the extensions again were found to be very few in number and comparable to those found in 1927. It thus appears that even when extensions occur this cannot be used as a sure sign of the presence of viable *B. amylovorus* (see Fig. 9), and that such extensions may be due to secondary invaders. *Sphaeropsis malorum* and other microorganisms very frequently follow-up the work of the fire blight pathogen.

Attempts at Isolating the Pathogen from Overwintered Material

The results of some of the isolations attempted in 1927 have already been presented; the remainder of those conducted at that time, about 50 in number, were all negative. As the numbers involved were not particularly impressive, it was decided to make a concerted effort the following year to determine the percentage of cases in which the pathogen may have overwintered. For this purpose 840 different isolations were attempted from various aged limb and twig blight and from both apple and pear throughout the winter and early spring. Out of this number 15 successful isolations were obtained, or slightly less than 2 per cent. The procedure used in each case was as follows. The material was washed in water, macerated with sterile knives in a petri dish containing nutrient broth, and kept over night. The next day the suspension was injected hypodermically into young, succulent Bartlett pear shoots, growing in the greenhouse. If a successful infection was obtained, this fresh infection was then carefully washed, treated with a surface disinfectant, macerated in sterile water, variously diluted, and used for a series of poured plates. The developing colonies were then grown separately in tubes of nutrient agar and nutrient broth and reinoculated into young pear shoots. If infections were again obtained the proof for the presence of *B. amylovorus* was considered substantial. This technique, in the writer's experience, is far more apt to indicate the presence of live bacteria of *B. amylovorus* than one in which an attempt is first made to isolate the organism directly from the diseased overwintered material. While the method used is very comparable to that employed by some pathologists in attempting to isolate *B. amylovorus*, some recent investigations conducted by the writer and his associates throw considerable doubt on its validity. There is a possibility that with other methods the numbers of successful isolations would be materially increased. Nevertheless, while the gross figures obtained are to be questioned, they do show the numbers of overwintered cases to be found in Arkansas,

Fig. 15. Holdover blight on Grimes Golden gathered near Farmington, Arkansas, July 20, 1929. Note the old, fungus-infected, blighted twig to the right and the new blight in the subtending limb. This canker served as the source of a cone-shaped area of infection. About natural size.

particularly on apple, when ordinary isolation methods are employed. The dates of isolation, type of tissue utilized and other notes are presented in Table 2.

An inspection of Table 2 will show that quite a few of the successful isolations were obtained in the early or mid-winter season and the criticism may well be made that these do not offer definite proof of overwintering. The assumption made by numerous investigators and backed by experimental evidence that *B. amylovorus* is more resistant to cold than to heat suggests that this criticism is not as well founded as it may appear

TABLE 2. SUCCESSFUL ISOLATIONS FROM PARTLY OR FULLY-WINTERED MATERIAL.

Dates when material for successful isolation was gathered	Kind of host[1]	Age and type of tissue
Nov. 29, 1927	Maiden Blush apple	One-year old twig with an indefinite, blistered margin.
Dec. 5, 1927	Yellow Transparent apple	One-year old twig with an indefinite, blistered margin.
Dec. 9, 1927	Yellow Transparent apple	One-year old twig with a definite margin, delimited by a transverse crack.
Dec. 14, 1927	Maiden Blush apple	One-year old twig with a definite, shrunken, but uncracked margin.
Dec. 20, 1927	Maiden Blush apple	Two-year old limb with a definite, uneven, cracked margin.
Jan. 6, 1928	Jonathan apple (near Bentonville)	One-year old twig with an indefinite, shriveled, uncracked margin.
Jan. 11, 1928	Ada Red apple (near Bentonville)	Two-year old limb with a definite, swollen and cracked margin, discoloration of tissues extending below the margin.
Jan. 16, 1928	Yellow Transparent apple	Two-year old limb with a definite, irregular, cracked margin.
Jan. 31, 1928	Jonathan apple (near Bentonville)	One-year old twig with a rough, uneven and indefinite margin.
Feb. 20, 1928	Kieffer pear	One-year old twig with an irregular but definite, cracked margin.
Feb. 27, 1928	Kieffer pear	One-year old twig with a slightly irregular but cracked margin.
March 13, 1928	Maiden Blush apple	Two-year old limb with a very rough, irregular, but uncracked margin.
March 21, 1928	Jonathan apple	Two or three-year old limb with an indefinite, uncracked margin.
March 21, 1928	Jonathan apple	One-year old blighted wood extending into two year-old, with a blistered, partly peeled and cracked margin.
March 27, 1928	Jonathan apple	One-year old twig with a definite, partly cracked margin.

[1]All material with the exceptions noted was gathered at Fayetteville.

to be. On the other hand, the sort of evidence that has been used for substantiating this claim involves pure cultures growing on liquid nutrient media or on solid ones, the main bulk of which consists of hydrophilous colloids. If the organism is as sensitive to dry conditions as it is assumed to be by various workers, then, it is conceivable that winter drouth may be just as fatal as a summer one, and there is considerable evidence that some of the so-called winter injury of various plants is due to lack of available moisture. With this in mind, it may be questioned whether claims for cold resistance in *B. amylovorus*, based as they are on trials with artificial culture media that are suffused with water, are indicative of the natural field behavior of this organism.

Aside from any significance that may be attached to the successful early winter isolations as evidence for overwintering, there can be no question that the pathogen has succeeded in doing so in a number of cases. The growing season in this

Fig. 16. Artificially infected Bartlett pear shoots by means of a watery spray containing *B. amylovorus*, in the two upper leaf clusters, the infections having commenced on the leaves, near the petioles, whence they spread downward into the stems. Note droplet of ooze near the base of stem at the left. Lower figure—healthy shoot for comparison, which had been sprayed with sterile water and kept in the moist chamber for 48 hours, comparable to those inoculated. (Photographed seven days after inoculation.)

region, as far as common pomaceous plants are concerned, very frequently begins in early March, as is indicated in Table 1. In 1927, for example, Kieffer pear blooming is noted as having

begun on March 7 and it will be remembered that prior to blooming there is always some leaf development in both pears and apples. There can be little doubt, therefore, that some of the data shown in Table 2 present conclusive evidence that the organism over-winters in very small, one-year old twigs of both apple and pear (see Fig. 10), as well as in older and larger limbs. While this may be considered conclusive, does it offer any definite evidence that such twigs and limbs served as centers for inoculum which produced the first spring infections? While such evidence has been commonly offered as indicative of sources of spring inoculum, the fact remains that working on an entirely different hypothesis evidence was obtained indicating that the organism may be present within the tissues and may be virulent as revealed by artificial infection experiments, without giving any outward indication of its presence, neither by producing blight symptoms in the neighboring tissues nor by producing infections in other nearby organs and tissues.

In the course of making daily observations of any possible oozing or other signs of activities of various cankers, special attention was given in the late winter and early spring of 1928 to a large, discolored area, representing the margin of a body canker of a Kieffer pear. In 1927 blight had completely killed the top of the main shoot of this tree for a distance of about eight feet, and while this was going on notes were being kept of the rate of death as seen by outward signs of disease, including the date and the distance of the last advance. The advancing margin was marked with a wax pencil so that it was possible to keep fair records of the amount of blight developed at any particular interval of time. In this way the tree itself gave a clearly discernible record of the rapidity of blight as well as the date of the abeyance of disease. The margin of this canker remained fixed throughout the winter and spring of 1927-1928, and being about five feet above the soil line could be easily and carefully inspected without much difficulty. As no oozing or other signs of activity of this canker had been noticed, in spite of its promising outward appearance including its dark-brown color and lack of cracking or blistering, it was decided to attempt isolations and inoculations from various parts, including regions of blighted bark several inches within the margin, from diseased and adjoining healthy bark at the margin itself and from a perfectly healthy leafy shoot. The latter, consisting of an elongated twig about one-half inch in diameter, had been established the previous season and had made good growth including bud formation prior to the envelopment of its base by the encroaching blight. It appeared perfectly normal the following season and its leaves as well as its bark including the basal portions, showed no signs whatever of blight attack. Several similar twigs were noted within the margin of this canker. As histolog-

Fig. 17. Natural infections on Yellow Transparent apple leaves and shoot showing droplets of ooze along petioles and on subtending twig. (Photographed April 27, 1927.)

ical studies of blighted wood had frequently shown bacteria to be present within the ducts of the xylem when they could not be found in the cortex, as will be detailed, the possibility appeared that the pathogen may actually pass upward or downward in some of the ducts without calling forth any disease symptoms. In accordance with this theory, the leafy shoot mentioned previously was severed and part of it, about 5 inches above the base, was used for inoculation experiments. This was carefully

washed at first, then macerated in nutrient broth and otherwise handled aseptically. After standing for 24 hours the broth-suspension was inoculated hypodermically into young Bartlett pear shoots and produced typical blight including the production of ooze on leaf petioles. The twig was gathered on April 12 when no signs of spring blight had as yet appeared, and the greenhouse inoculations made from it were first noticed on April 21. From one of these blighted shoots the organism was re-isolated and its pathogenicity again determined by inoculating into pear shoots growing in the greenhouse.

This finding of blight producing bacteria within a healthy-appearing shoot several inches removed from formerly blighted material is of course insufficient for any generalization, and, while other, but less clear-cut data are available, which seem to indicate that this is not an anomalous phenomenon, it is offered primarily to show that the mere presence of blight producing bacteria within overwintered limbs and twigs is not sufficient evidence for the theory that the first blight is started from oozing or surface-borne inoculum derived from such material. Not a single sign of fresh blight was noted on this tree until several weeks after the disease had been found on nearby apple trees, and the indications were excellent to show that when the blight finally appeared it had been brought in from some other source.

By calling attention to the lack of previous evidence for the connection of first spring blight with hold-over blight, there is no intention of rejecting the possibility that such hold-over blight may act as a source of inoculum at one time or another during the current season. Indeed, there is some evidence to show that this is true, but there are also other possibilities. The evidence that would connect at least part of the current infections with hold-over blight consists of two different kinds, first, the common occurrence of blight close to previously blighted material (see Figs. 11, 12, and 13), and, second, the activity in some cases of old blighted tissues in the following spring and summer.

When a badly blighted tree is examined it is extremely difficult to trace the infections to the one or more possible sources from which the blight had started. Even where cones of infection are to be noted as Brooks *(5)*, Miller and Keitt *(24)*, and Miller *(23)* have described for Wisconsin apple orchards and as the writer has found in Arkansas pear and apple orchards, it is not at all easy to trace the infections to some one source for the reason that, by the time the cone-shaped mass of newly initiated blight is observed, there usually has appeared an epidemic of blossom and twig blight scattered promiscuously over the trees which seriously interferes with the tracing to any hold-over canker. Even where the new infections all center around or

Fig. 18. Three Kieffer pear leaves (above) infected with blight following a very severe hail, wind and rain storm. These leaves were attached to a tree which harbored considerable twig blight and following the storm showed a large number of leaves with fair-sized blackened areas, whereas nearby pears, which had been free from blight, showed leaves just as badly injured (lower leaves) but without the blackened areas noted in the infected tree. Bacteria were observed in these areas and *B. amylororus* cultured from them. (Photographed May 13, 1927.)

taper to an old blighted limb or twig, the evidence is by no means conclusive that this new blight developed prior to any other blight unless the particular hold-over has at least been definitely found to be active early enough to account for the appearance of the first blight, given an incubation period of about 10 to 14 days. The writer has found, in artificial infection experiments conducted on pear and apple trees out-of-doors in

the early spring, that when successful infections were obtained no less than 10 and often as many as 14 days intervened between the application of the inoculum and the first signs of blight. This is quite comparable to the period that Brooks *(5)* has reported for Wisconsin. As the spring season advances the period of incubation is perceptibly shortened so that in early May the period in artificial infections, and presumably in natural ones, is reduced to five to seven days or even less under very favorable conditions. All of this contributes to the difficulty of tracing the infections. Nevertheless, in several instances unmistakable cases of overwintering plus dissemination under natural conditions were found. In a 48-acre Jonathan orchard located near Farmington, Arkansas, a number of blighted trees were noted in which the disease appeared localized, infected flower clusters, twigs and limbs all being bunched together, frequently at or near the tops of the trees. When these infected centers were carefully examined, a blighted canker of the previous season (see Fig. 14) was invariably found above or near the center of the infected area. These overwintered cankers showed unmistakable signs of activity; the margins had extended, producing newly killed areas of bark which by its color and sometimes by its oozing could be clearly distinguished from the older, lighter colored, more or less withered blight. At times the older portion of the canker was represented by a markedly constricted area which was studded with pycnidial fruiting bodies of the black-rot fungus, *Sphaeropsis malorum*, the constriction evidently having resulted from the localized death of the bark in the previous season. The infections in these cases had not prevented the growth of the adjoining parts above and below the canker, so that the constricted region represented a part of the limb which had ceased growing while the neighboring parts continued to grow. In contrast to this, when new or young infections are found, no such constrictions are noticeable, variations in size of diameters between diseased and healthy parts being slight or none at all. Consequently when a canker in the form of a very marked constriction is noted, and when it is further found to have extended into the much thicker, adjoining tissue resulting in fresh blight, the conclusion appears inescapable. This, coupled with the fact that much blighted material centered around such cankers, makes it logical to assume in these instances that the blight was initiated by such cankers. The writer looks upon this as substantial evidence for the carrying-over of the blight pathogen (on apple in this instance) from one season to another, but is not prepared to accept it as evidence for the initiation of the first spring blight. In the particular cases cited the cone-shaped infected areas had not been observed until July 19, after the main epidemic of blight for that season had developed.

Fig. 19. Kieffer pear leaves and shoots showing oozing of the latter and the type of indirect leaf invasion along the midrib of leaves resulting from the migration of the pathogen from the shoot by means of the petiole. The same sort of leaf spots may also be produced by direct inoculation, applied as a spray, to the leaves. (Photographed April 18, 1927.)

Why had the centers or cones of infections in this orchard occurred near the tops of the trees in most instances? The answer is to be sought partly in the lack of proper pruning and partly in the favorable situations for water-borne dissemination of bacterial ooze. It is, of course, much easier to see diseased limbs near the base of the tree and much easier to remove them than in parts that are higher up and hard to reach. These trees were large, 20 years old and fully grown. Pruning in this part of the country is usually practiced in late winter or early spring, which also makes it more difficult to detect diseased wood. From this point of view, pruning out of fire blight could best be done in the fall when the leaves are still attached.

It may also be of interest to note that this large Jonathan orchard is located in a region where pear trees had been removed, and when the orchard was under observation in 1929 the nearest pear trees, five in number and known to the local people, were located more than 1½ miles from this orchard with large blocks of apple orchards interspersed between, a number of which showed a very small amount of blight or none at all. As compared to the season of 1920, two years before any concerted effort had been made in the removal of pears, the season of 1929 revealed just as much, if not more, blight. In the earlier year blight had been quite serious in this Jonathan orchard and has persisted undiminished since that time. The probability is that, as far as this orchard is concerned, pear trees have had very little to do either with the overwintering of

the pathogen or of disseminating it after the blight had been initiated.

In addition to cone-shaped areas of infection found in the Jonathan orchard noted above, a number of other similar cases have been found in other orchards involving other varieties of apples (see Fig. 15) as well as pears. In each instance infections could be traced to hold-over cankers which resided near the apex of the infected areas. All such hold-over cases in apples involved limbs that were not more than 1½ inches in diameter, most of which were smaller. It is well known that in apples infections do not involve the large limbs and trunks as frequently as they do in pears. As a consequence, if the bacteria are to over-winter on the apple, they may be expected to do so on relatively small limbs and twigs. To some extent this makes it harder to combat blight on apples. If the disease is to be properly controlled, it is obvious that all blighted wood, including the small twigs, must be removed. The writer is convinced that the emphasis placed at present on large limb and trunk cankers as special seats for carrying the pathogen from season to season is grossly exaggerated.

Cytological and Histological Studies of Blighted Material

As a background for a satisfactory understanding of this disease and the means by which it is perpetuated, it is obvious that a clear cut picture must be had of the host cells and tissues which are involved, of the manner in which these cells respond to the presence of the parasite, and of the host cells and tissues which may serve as a possible source of overwintering. For example, if it is true that only cankers with an indefinite and unconfined margin are primarily responsible for the overwintering of the blight producing bacteria, as has frequently been assumed, then, it is quite obvious that no particular attention need be paid to the other type of canker, as far as blight control is involved. This in turn may be considered as resting upon host reactions in which the diseased parts are either cut off from the healthy tissues by a layer of cork eventuating in a clearly defined, often cracked margin, or by no such delimiting layer, in which case the organism, according to the prevailing opinion, may be expected to perish in relatively short order and to play no part in perpetuating the disease. Again, if the disease producer is strictly confined to the bark parenchyma and is only rarely to be found in the inner tissues including the xylem, as is also commonly assumed to be the case, then, it is quite obvious that the application of germicides to the surface of cankers or the excision of affected bark may with more certainty be expected to kill or remove the pathogen than if it were deep seated within the tissues. Obviously a cytological and histological study of blighted host tissues is of fundamental importance.

While a number of investigators have dealt with various phases of this question, it is surprising what little concerted effort has been made to study diseased tissues. A few more or less general statements, often repeated in numerous publications, are to be found in the literature, usually relative to the cortex being the main seat of the disease, and there appear to be only two investigators who have attempted to study diseased

Fig. 20. Duchess pear blossom cluster showing artificial infections obtained by a water spray of *B. amylovorus* when the blooms were tightly closed. note the dark, infected discolorations on the receptacle walls and on the adjoining calyx lobes. (Photographed three days after the inoculation.)

tissues with any degree of adequacy, Bachman *(3)* and Nixon *(25)*. Miss Bachman confined her studies to paraffin sections of artificial infections in diseased blossoms, fruit, and succulent shoots, and evidently made no effort to study hard, woody tissues at all. Nixon, also dealing with paraffin sections, worked with woody materials as well as with succulent ones, but he seems to have been more interested in the bacteria than in the host tissues. This, of course, is not surprising to anyone who has attempted to study woody tissues that have been through the

parafin imbedding and the subsequent staining process. By this method the sections are likely to be so torn and broken by the knife, so badly stained, or so devoid of the pathogen that it is extremely difficult to obtain good histological views of any fair proportion of the diseased and healthy tissues. The best that can be had, as illustrated by the drawings of Bachman and Nixon, by the few photomicrographs of D. H. Jones and of one or two drawings and photographs in text books, consists mainly of views of bits of succulent cortical tissues, or a few cells of fruit pulp or of an isolated piece of woody bundle. Good photomicrographs of any kind and especially of diseased twigs and limbs, are extremely rare, the one given by Smith (*34*, Fig. 286, p. 371) being the single possible exception.

Burrill and Waite made a few statements relative to diseased tissues. The former *(6)* gives the following account: "In very young tissues, such as the tips of apple tree shoots, all parts except the epidermis seem to be equally invaded, but in older limbs the bark parenchyma, or the outer layer of living bark, is the first and usually chief seat of the disease. The bast . . . is not affected. . . . Sometimes invasion through a layer of bast is gained by way of medullary rays. Contrary to the usual opinion, the cambium . . . is by no means the seat of the disease." He finds no evidence of the progression of the disease in the wood, and the discolored xylem within diseased areas is not explained, although he does attempt this for the parts above the diseased portions. As to cellular changes, he considered the disappearance of stored starch as being the most conspicuous, and utilized this supposed reaction in giving the specific name to the organism. "*The cell walls were not dissolved or altered, in any way*, (his italics), except by the staining, which sometimes takes place through oxidation, in the later stages of the disease. If there be any exception it is confined to the thin walls of very young cells, which soon shrink and become, by drying, much distorted." He does not consider the bacteria capable of being carried in the "circulation of the fluids of the tree" and attempted to explain the passage of bacteria through the tissues on the basis of cell wall penetration, nothing whatever being said about intercellular migration. Waite has published a few brief statements on the internal pathological phases of this subject. "The most important parts of the tree killed by the blight are the inner bark and cambium layer of the limbs and trunk." *(44)*. The microbes of pear blight "live and multiply in the liquid contents of the cells of the pear appropriating its juices to their growth. They have the means of breaking down the partition between the cells and thus spreading throughout the softer structures. This seems to be due to the dissolving action of their secretions on the cell walls. They corrode small channels through the tissues. These often break out at the surface and

Fig. 21. Artificial infections on Kieffer pear pedicels and calyces as a result of spraying with *B. amylovorus*. The pedicles of the upper row and the lower left are mostly killed clear to the base. Note the droplet of ooze at the base of the discolored calyx lobe in the lower-middle bloom. (Photographed four days after inoculation.)

the gummy mass of germs exudes as a small drop" (43). The germ "primarily attacks the fleshy portion of the twigs and branches and avoids the fibers or vessels which conduct the

water. . . . Perhaps during a particularly moist time, as for example, while a thunder storm is passing or in warm rainy or cloudy period for a day or so, when the tree becomes gorged with sap from root pressure because of the transpiration from the leaves being diminished, the microbes in the fleshy bark overflow into the vessels, choke them up, and kill them, and as a result when the sun comes out and the dry weather returns, such trees collapse and the foliage and all the parts above the blight die. . . . This collapse of girdled branches and the dying of branches already practically killed by the bark being dead, during rainy periods or thunder storms, had led people to think that the blight is more rapid than it really it" *(46)*. "When the blight is working in the tissues, it invades the vessels of the bark, the intercellular spaces, and besides often breaks down, in its progress, the little pores or channels or sometimes large lenticular spots, which become filled with a mass of gummy matter" *(47)*. "The more mature twigs and branches are killed mainly through destruction of the bark. Under extreme conditions the germs enter the sap wood and they regularly enter the very young sap wood on the twigs" *(48)*.

D. H. Jones (*21*, p. 12) considered the inner bark as the main seat of bacterial activity, as far as twigs are concerned, although the proof for this, his figures 11 and 12, are based on sections of diseased fruit pedicels. Of larger limbs he merely states that the disease progresses in the bark and that "the germ lives in the tissue cells of the bark" (p. 19). O'Gara *(26)* found bacteria within the rich sapwood of the Bartlett, Howell, and other pears and in that of the Spitzenberg apple. Bachman *(3)* made a careful histological study of artificially infected pear blossoms, of young shoots of apples, pears and plums, of pear seedlings, and of pear and apple fruit. She made no effort to study the disease in natural infections or in hardened woody tissues. Her chief contributions consisted in finding of bacteria in intercellular spaces of cortex of fruit pedicels and of fruit, as well as the detection of bacteria in the xylem tubes of shoots and seedlings. Stewart *(35)* partly confirmed Miss Bachman's observations, especially those pertaining to intercellular spaces, although he also found the bacteria to be present to some extent within the cells. He found the cortical parenchyma to be most severely attacked and sometimes the cambium to be affected. Also, according to Stewart, and as Burrill had previously found, the pith and xylem of young shoots may be invaded.

The more recent work of Nixon *(25)* deserves special attention. By means of needle punctures he inoculated water sprouts[2]

[2]The species and varieties are not given, but from a general statement made on another page must have included one or all of the following apple varieties: Early Transparent (Yellow Transparent?), Stayman, Williams, and Ben Davis. The present writer knows nothing about the susceptibility of the Williams variety but of the others mentioned the Transparent apple is the only one that is markedly susceptible under natural field conditions.

and one hour after inoculation the small clumps of bacteria, introduced on the needle, were seen to be imbedded in a jelly-like substance, referred to as a zoögloeum, which (p. 9) "without exception is first developed in the cortical region about ten cells from the epidermis". From this point it advances by means of pseudopod-like projections through the twig in all directions radially, tangentially, and longitudinally, the paths being the intercellular spaces of the cortex. "From this path the bacteria may extend radially . . . until the surface is reached, and . . . until the pith is invaded. This invasion, however, may well be characterized as secondary. Most of the evidence seems to indicate that true intercellular migration occurs only in the optimum region (*i. e.* cortex) and that invasions of the less favorable tissues should be regarded as secondary effects. . . . Sometimes, under especially favorable conditions, the bacteria multiply in the early zoögloea mass until the wound cavity is gorged. It is under such conditions that regions other than the optimum are invaded, *e. g.*, the intercellular spaces of the pith, the vascular bundles, the phloem, etc." However, he finds that tissues other than cortex may be invaded by means of zoögloeal strands originating in the cortex. "Toward the interior the organism ultimately traverses radially most of the intercellular spaces of all the tissues of the young stem. The last tissues to be invaded are the cambium, the xylem and pith, and these are rarely seriously attacked." He considers this a different sort of migration from that which occurs in the cortex. "It always seems to be associated with the presence of large masses of bacteria in normal intercellular spaces or in schizogenous cavities, suggesting that pressure or 'mass action' may play a considerable part in this type of migration". The cortical invasion is considered to be much more rapid, while the latter is slow and involving various tissues for only short longitudinal distances. He notes that "cells are frequently found completely surrounded by bacteria without showing any visible changes" and that the first visible effect on the host cells appears not before 48 hours after inoculation "depending somewhat on the condition of the tissue. . . . This effect might be considered as being the result of a loss of water. There is first a slight, probably toxic plasmolysis, followed finally by the complete collapse of the proto-

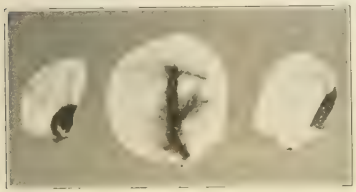

Fig. 22. Kieffer petal infections four days after inoculation. Blooms kept for 48 hours after inoculation in a moist chamber made of cloth sides, maintained in a greenhouse. The inoculum consisted of a spray of *B. amylovorus* in a water suspension.

plast but never its dissolution. . . . The cell-walls finally yield to pressure, undoubtedly exerted upon them by the bacterial mass, and larger or smaller schizogenous cavities appear, formed by the splitting and separation of the cell walls. . . . There is never any indication of the dissolution of the cell-walls or cell contents. Neither is there any evidence that the bacteria enter these cells."

Nixon regards the bacteria within these "schizogenous" cavities and in the immedately adjacent spaces as being short lived, finally disintegrating completely and "leaving no trace of their presence in the dying tissue of the young sprout." But in the zoögloeal projections that have extended downward into the older tissue "the bacteria are found to undergo a transformation in size and apparently in functional powers." They are shorter, stain deeper and "tend somewhat to be oriented in the same direction. . . . In this form they can be found in the intercellular spaces of all regions of the basal part of the water sprout and adjacent limb, except the hard bast, xylem, pith, and perhaps the cambium. Their intricate ramifications are most conspicuous in the region of the medullary rays. . . . At various intervals throughout these regions the bacteria are found inside the host cells, a condition which is not found in the typical zoögloea stage. . . . They ultimately destroy the protoplast with all its contents, together with the cell-walls. Frequently, they invade groups of adjacent cells and, breaking them down entirely, form lysigenous cavaties of various sizes. . . . These cavities may be found in any of the tissues mentioned above, but are most prevalent in the cortical region, phloem parenchyma and medullary rays." While "the zoögloea stage and the formation of schizogenous cavities are found most commonly in the young succulent parts of the water sprouts . . . and the reduced stage, with the accompanying lysigenous cavities, occurs with greater regularity in the tissues of the older limbs and trunks, the reverse condition, however, has been observed occasionally."

With the formation of "lysigenous" cavities Nixon finds the "reduced form" of bacteria distributed within the lumina of "certain cells, particularly those of the phloem parenchyma, cortex, and to a less extent, those of the phloem ray cells." In this stage they are imbedded in a gelatinous substance "unusual in abundance and density. . . . The bacteria then aggregate themselves into a core-like center, often arranged in spiral series . . . and this development continues until the entire mass is rounded up and the individual bacteria are no longer distinguishable." This he interprets as being stages in the formation of cysts which he found to exist in living cankers, particularly of Transcendent crab, and which he further believes is the stage responsible for carrying the bacteria over winter. As proof he offers the observation that when such masses are "teased out of

fresh tissue," mounted in water, and inoculated into young apple shoots, typical blighting ensues. He, however, made no effort to isolate single cysts for inoculation purposes or to culture *B. amylovorus* from them. Until this is done there is obviously no assurance that the infectious material contained in his

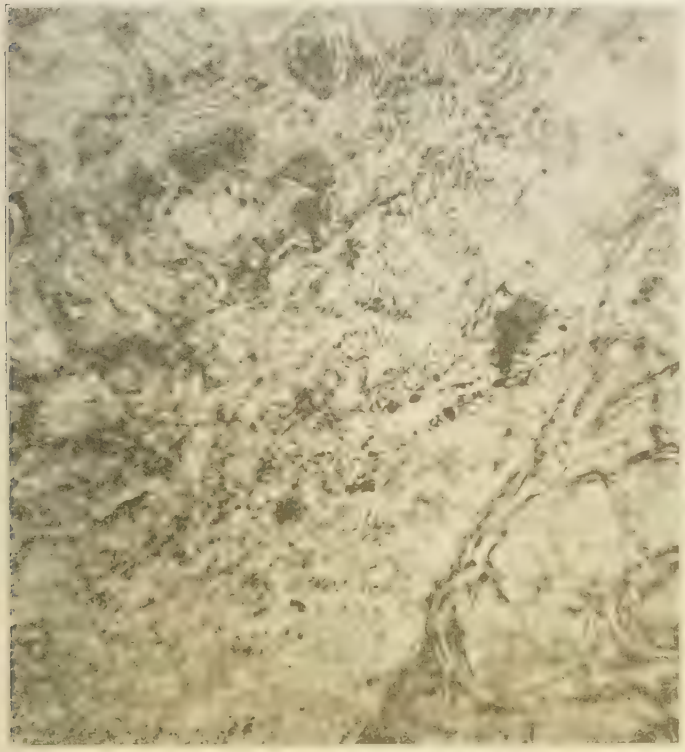

Fig. 23. Surface view of an artificial infection on a Duchess pear petal showing bacterial mass within a fold. Note surface corrugations of petalary tissues on both sides of the bacteria, indicating the superficial position of the bacteria. Magnified about 600 times.

inoculum did not arise from some source other than "cysts." As will be shown later, bodies very much resembling Nixon's cysts are commonly found in diseased material and are often non-infectious. Aside from this there can be no question that Nixon's careful studies of the migration of the bacteria has given us a tangible basis for an explanation of bacterial move-

ment within diseased tissues, even if one rejects his idea that it is comparable to the movements of pseudo-plasmodia.

The histological and cytological studies that are here to be detailed involve principally the following: first, floral infections, especially the petals; second, leaf petioles; and, third, succulent and woody twigs and limbs of varying ages. Attention must first be directed to the fact that by the methods commonly used it is extremely difficult if not impossible to prevent the loss of most of the bacteria in infected tissues. By any method of killing and fixing, whatever the killing fluid might be, there is almost always a diffusion of great numbers of bacteria from the blocks of tissue into the killing solution. The writer has used a number of different killing agents including Flemming's, Carnoy's, Gilson's, chromo-acetic acid, formolacetic, hot alcohol, hot and cold formaldehyde and others, and invariably there was a loss of bacteria from the tissues in such numbers as to cloud the fluid. The use of a freezing microtome was also of no advantage in this respect. Unless some method is devised which will prevent this, it can be said that in any fresh infections, as well as in older ones which must be cut, there is bound to be a considerable loss in the amount of bacteria which is present within the tissues and that this loss will occur in at least two different stages of the process –in that of killing, and in that of sectioning. This is particularly true of stems and petioles. In spite of the fact that investigators have usually said nothing about this difficulty, it is quite obvious that whenever it occurs it becomes extremely difficult to determine the changes that have been brought about in the diseased tissues by the bacteria. In the absence of the pathogen, a comparison between diseased and healthy tissues is subject to such diverse interpretations that conclusions are almost impossible without considerable "hedging". While gross histological changes, such as cavity formations, are readily discernible in the absence of the pathogen, the finer cytological changes are likely to be overlooked or misinterpreted. For this reason the writer has had to reject considerable work of his own as well as of assistants, which involved killed and imbedded preparations, and the studies to be reported were made largely from material which was cut in a dry stage on a sliding microtome without any previous imbedding, or in the case of soft, succulent tissues with an ordinary sectioning razor. This by no means relieved all the difficulty mentioned, but it resulted in preparations which, as a whole, were far superior to the others and it also paved the way for studying whole organs in which there could be no question about the bacteria remaining intact within the tissues.

Cytological and Histological Studies of Floral Infections[3]

The great difficulty encountered in preserving the bacteria within diseased tissues made it necessary to search for methods and material where this might be overcome. As former work *(31)* had indicated that infections may be obtained in blossoms by simply spraying them with water suspension of bacteria, this method was utilized on various plants and plant parts, including

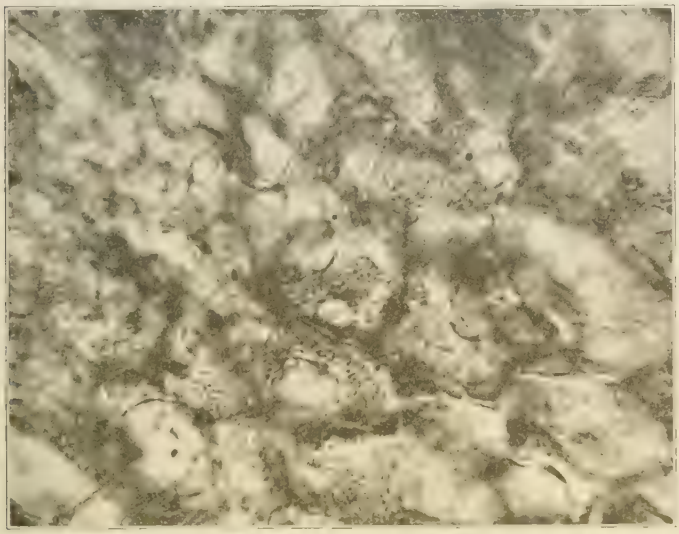

Fig. 24. Duchess pear petal mounted *in toto* showing bacteria within the tissues. Note the extraordinary number of bacteria observable, the compactness of the bacterial strands the partial obliteration of cell walls and presumably of middle lamellas, the presence of bacterial strands within the walls and surrounding the protoplast but not within the latter. For wall destruction and for presence of bacteria within walls note especially Fig. 25. Magnified about 800 times.

young leaves and blossoms, with the hope that by so doing material would be made available for study in which the bacteria might not diffuse out of the diseased structures as readily as in twig or limb infections. Also in these tests an explanation was sought for certain types of blossom infections noted under field conditions, as previously reported *(30)*, in which blight occurs in blooms that are tightly closed and in which there is very little likelihood of transmission by pollinating or nectar-seeking insects. Are such infections extensions of adjoining

[3]In a preliminary note in Science, Vol. 70, 329-330, 1929, the writer calls attention to petal infections in which the bacteria can be clearly observed without sectioning.

blighted tissues or may they be induced by water or wind-borne inoculum? Are nectaries the only floral tissues which permit blossom invasion or may other parts serve the same purpose? Then again in studying the host range of *B. amylovorus (31)* the writer has been greatly perplexed by the fact that this organism, according to most authorities, could induce infection through only one type of natural opening, the nectary, in spite of its exceptional wide range of hosts. What prevents it from entering and infecting through stomata or water spores, especially in tissues that are known to be highly susceptible to blight?

A great number of infections were obtained when a heavy bacterial suspension in water was applied to a cluster of very young Bartlett pear leaves attached to growing plants kept in the greenhouse or to clusters of pear flower buds borne on twigs which were obtained from nearby out-of-door pear trees, and keeping such sprayed parts within a cloth-enclosed moist chamber for 24 to 48 hours, alongside of checks sprayed with sterile water. (See Figs. 16, 20 and 21.) Within 24 hours after inoculation many small water soaked areas were to be seen on the young partly folded pear leaves particularly around the lower parts of the midrib, frequently flanking this organ on one or both sides. If the infections occurred within the lamina, some distance removed from the petiole or lower parts of midrib, then they remained small, localized, and of no particular significance as far as damage is concerned. But when they occurred near the petioles, then they were quite likely to run down into these organs and eventually involve the whole shoot. (See Fig. 16.) There can be little doubt that the original invasion occurred through stomata, and this is in substantial agreement with the very recent work of Tullis *(38)* on apple leaves, as well as with Miller's *(23)*.

It may be worthwhile to call attention to the fact that natural leaf infections in the field are of three different sorts. First, leaf infections resulting in direct invasion through natural openings, often occurring along the base of the midrib and extending downward into the petiole (see left hand leaf of Fig. 17). This is very easily confused with indirect invasion which is to be shortly described. Artificial infection experiments with spray applications of bacteria have resulted in very similar symptoms (see Fig. *30* p. 69). Second, leaf infections resulting in direct invasion through wounds (see Fig. 18). This, unlike the first which mainly occurs on very young leaves, may be found on both old and young leaves. Third, leaf infections resulting in indirect invasion (see Fig. 19) in which the shoot or stem becoming infected, transmits the invader from stem to leaf tissue. Most leaf invasions observed under natural conditions are of this kind.

Comparable to artificial infections on young pear leaves

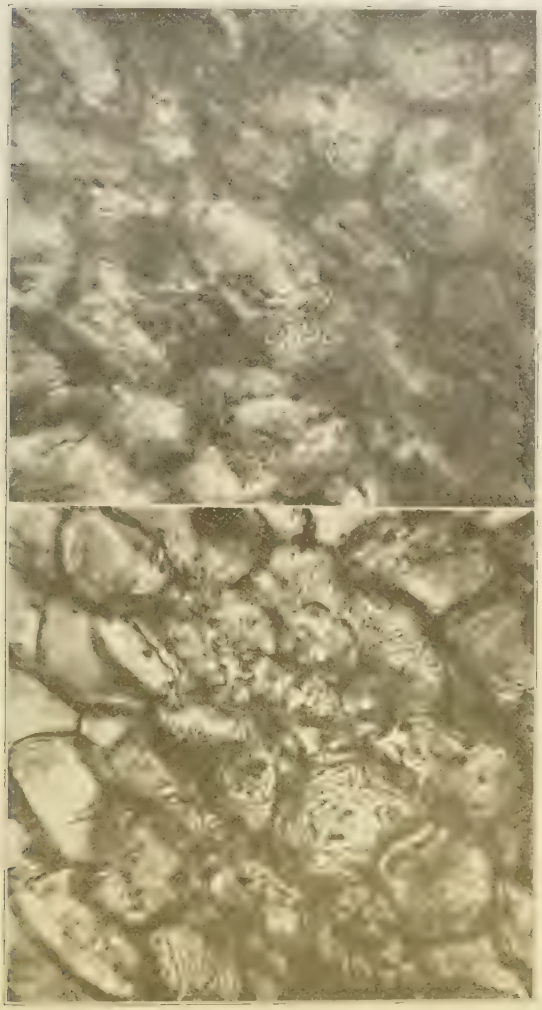

Fig. 25. Duchess pear petal mounted *in toto* showing bacteria within the tissues comparable to those shown in Fig. 24. For comparison between diseased and nearby healthy tissues note lower figure with the healthy portion at the left. Magnified about 800 times.

were those obtained in different parts of young pear flowers. Upon the latter they developed on the peduncles, walls of receptacles, on the calyx lobes, (see Figs. 20 and 21) and on the petals. Those involving peduncle or receptacle infection were far more serious than the others, often spreading considerably, resulting in the more or less rapid death of the whole flower and occasionally running down to the spur, eventuating in the death of the entire flower cluster. But when the tips of the calyx lobes, or when the petals became infected, the resulting discolorations invariably remained localized, although a droplet of ooze could sometimes be found clinging to an infected calyx lobe (see Fig. 21)

For studying the activities of the pathogen within diseased tissues, the petal infections are unsurpasses. Localized as they are (Fig. 22), they permit the mounting of a whole infected region plus surrounding healthy parts in a drop of water and being translucent permit remarkably clear views of internal structures without recourse to sectioning. This in turn enables one to study tissues in which the number of organisms remains practically intact.

As already reported, blossom infections on pears may be obtained within 24 to 48 hours after inoculation. Apple blossoms have likewise been tried but for some unaccountable reason have not yielded petal infections, although three different attempts were made involving several hundred blossoms. It is possible that the apple petal with its anthocyan pigment is resistant to infection? Of the infections on pear petals two different varieties were used, Kieffer and Duchess, both of which yielded a number of infections involving about two per cent of the total number of petals present in the trials. From one of the artificially infected petals the organism was re-isolated, grown in pure cultures, re-inoculated into healthy pear shoots, resulting in the production of typical blight. How did the pathogen gain entrance into these delicate structures? Up to the present, natural openings have not been detected although a diligent search has been made. It is well known that petals of various plants possess such openings, though in very thin petals they may be vestigial, as stated by Eames and MacDaniels *(12)*. But in the petals here used the surface cells show mound-like projections and if openings are present they must certainly be very much smaller than ordinary stomatal ones. These mounds plus the intervening low places are embellished with surface sculpturings in the form of a parallel-running series of wavy lines or ridges. Neither have openings been found at the margins. Another feature characterizes these young, unopened pear petals, namely, the relative large number of more or less delicate folds or invaginations.

There can be no question that a number of the infections

on petals were obtained by means of wounds. These may be expected to appear frequently on such fragile organs, and there has been no difficulty in tracing infections in certain instances to these wounds. But in other cases such wounds have not been found in infected regions. Here it may be worthwhile pointing out that, unlike certain fungi, bacteria are not considered to possess the property of penetrating outer walls and hence must gain entrance by means of a wound or through some well defined

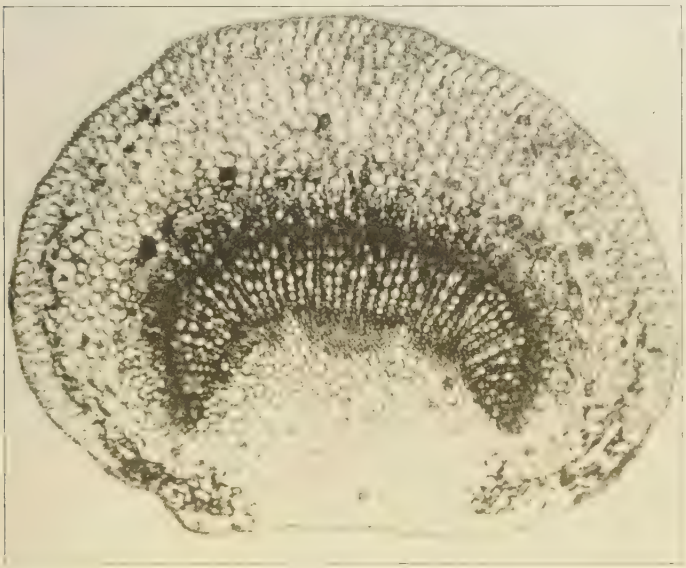

Fig. 26. Cross-section (free hand) of a freshly infected Bartlett pear petiole showing the extent of the necrotic regions within the outer cortex. Pathogen lost in the process of sectioning. Magnified about 80 times.

natural opening. It has been well established that some and perhaps all fungi capable of thus penetrating do so by mechanical means. The appresorial formation acts as a hold-fast for the penetrating plug or conceivably in other instances the fungus spore itself, by its ability to secrete a substance capable of holding it tightly to the surface of the host, acts as a mechanical lever. No such properties are known for individual bacteria. But what are the possibilities of a mass of bacteria gathered together in a small fold, making their way into the tissues by either "mass action" involving in this case the leverage engendered in the sticky mass adhering to the surface, or by the formation of cell-wall dissolving enzymes? It is to be noted that the

outer walls of these petals are by no means to be compared with the hard, cutinized walls of leaves. They are rather thin and so delicate that they frequently rupture during the process of floral expansion. In Fig. 23, such a bacterial mass may be seen in a small fold. The surface markings of the petal may be seen clearly on both sides of the pocket, while the tissue beyond this, particularly as seen in the lower right-hand corner, has sloped upward to such an extent that the interiors of the cells, not the surfaces, are in optical allignment. The particular infection here involved centered around this fold. The theory for penetration which has just been proposed is presented merely as a working hypothesis which is yet to be proved. There seems to be a great dearth of knowledge or lack of interest in the study of bacterial invasion of any sort in spite of its fundamental importance. The mere statement that a certain organism is capable of penetrating through wounds or through natural openings appears to be the beginning and the end of our knowledge of penetration. How do the bacteria penetrate the tissues after they enter the wound or substomatal chamber? Do the bacteria grow and multiply in the latter, and, if so, what is the source of their food? Unless one assumes the presence of an actively growing, rapidly multiplying bacterial mass as an infection nidus, how then can the rapidity of infection in *B. amylovorus* be explained? If such a nidus is assumed, then do the conditions within substomatal chambers vary sufficiently to account for infections occurring readily in young pear and apple leaves in contrast to older ones which, in spite of their susceptibility as seen in wound inoculations, remain practically free from stomatal infections? These petal infections will be further studied with the hope of obtaining some information on these points.

The bacteria having gained entrance into the interior by wound or otherwise, how do they spread and what are the effects noted on the host cells? Figures 24 and 25 show clearly that the paths of invasion are between the cells. This involves a great deal more than passage through intercellular spaces. A close inspection of these views shows strands of bacteria completely surrounding the protoplasts with very evident indications of destruction of parts of the cell walls. This destruction is so complete in some instances that the original outlines can only be conjectured. Obviously the bacterial strands have not only made their way along intercellular spaces, which in these organs are very few in number and small in size, but have, by the destruction of walls plus middle lamellas, manufactured passages for themselves through the walls. The pictorial evidence points clearly toward a chemical dissolution rather than a mechanical rupture. The most impressive part of these views are the enormous numbers of bacteria making up a strand, so tightly are they wedged together between cells or between protoplasts that

it would hardly seem possible for any more to exist without distorting the strands considerably. This latter possibility has not been observed, the strands always appearing neatly regular and taking the shape of the intercellular space or of the adjoining walls on one side and of protoplasts on the other. There is no suggestion whatever of mechanical push or strain in the host tissues.

In no case have the bacteria been observed within the protoplast. As already noted, they frequently make their way into cell walls and occasionally give the appearance of occupy-

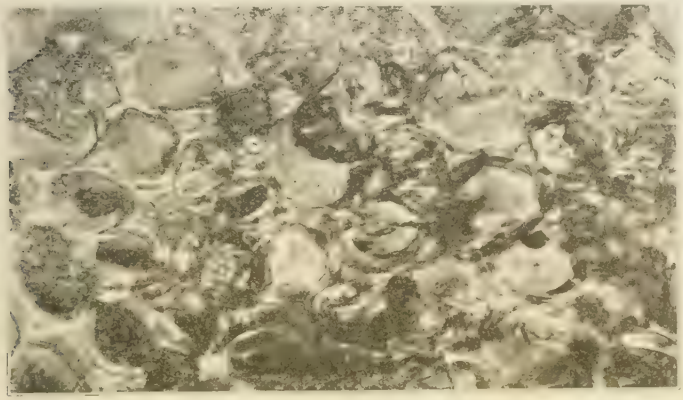

Fig. 27. Cross-section of freshly infected Bartlett pear petiole showing the partial and complete plasmolysis and discoloration of protoplasts within the cortex. Note the bacteria present within the cell walls of one cell near the center but not within the shrunken protoplast. Many of the bacteria from this section have probably been lost in the process of sectioning and mounting in water. Those apparently observable within protoplasts may reasonably be explained as either having been wafted there by the mounting fluid or as representing masses immediately above or below the protoplasmic linings. Magnified about 500 times. (See Fig. 28, cross-section of healthy petioles for comparison.)

ing the entire cell, but this may readily be interpreted as a top or bottom view of some particular cell with the protoplast unoccupied in some portion not in view. It will be shown later that this phenomenon also occurs in other types of infected tissues and organs. The figures also give a clear conception of the enormity of bacterial invasion. There is evidently a closely knit, compact wall of bacteria surrounding most of the cells within the infected region. The evidence is excellent that bacterial invasion has not simply involved the passage of a few strands which may impinge on one or more small areas of cell walls; there appears to be an invasion which completely surrounds the cells or protoplasts in three dimensions. In order to clarify the nature of the phenomenon here involved we may

compare the invaded region to a piece of Swiss cheese in which the bacterial mass represents the substance of the cheese while the protoplasts, or wells and protoplasts, represent the lacunae.

All this may be readily observed within 48 hours after inoculation when a portion of the petal containing the infected region is mounted in a drop of water and examined under the microscope. For photographic purposes infected petals were placed in 70 per cent alcohol, boiled for about five minutes, and then stained for about one minute in a very dilute, water solution of Ziehl's carbol fuchsin. It was found that when this material was mounted in water with the edges of the cover glass sealed with Canada balsam, the views were much clearer than in mounts in which a series of alcohols were used followed by xylol and mounted in balsam. For the finer cytological details the living, unstained material was found to be more satisfactory for study than the stained preparations.

The extreme rapidity of infection in these organs makes it difficult to find very early stages. While Nixon records that the first visible effect on the host cells appeared not before 48 hours after inoculation of apple sprouts, these petals, and as a matter of fact pear and apple shoots and leaves develop, under suitable inoculating and incubating conditions, well defined necrotic, water-soaked areas within 24 hours and by the end of 48 hours the infections on petals as well as pear leaves are very conspicuous. Since these petalary infections have up to the present been studied mainly in 48-hour old lesions, it is obvious that the initial stages remain still to be investigated. But, whatever these may show, there can be no question that the rapidity of growth and reproduction of the pathogen is totally beyond anything that has previously been postulated. This in turn must mean that the invader finds materials which it can metabolize in very short order. Where do these materials come from?

Water is undoubtedly withdrawn from the protoplast resulting in a marked plasmolysis, as has been well described by Bachman and others. But the protoplast itself is evidently not readily available for nutrient purposes, as can be seen in these photographs, and this also is in agreement with Bachman and Nixon. The delicate mesophyll cells which make up the bulk of the petals are large, relatively thin walled, with the protoplasts evidently highly vacuolated and surcharged with water. The protoplasmic lining next to the walls is very inconspicuous in normal cells, while the protoplasmic interior shows only a slight amount of granular material, some indeed showing none at all. In view of the fact that the killed, highly plasmolyzed protoplast is frequently observable within dead cells, it may be assumed that the complex nitrogenous compounds contained within the protoplasm are not used to any noticeable extent by *B. amylovorus*. This, however, does not mean that solutes in-

cluding soluble nitrogenous compounds, organic and inorganic, are not withdrawn from the protoplast. Indeed, the appearance of a diseased protoplast which in contrast to a healthy one is markedly conspicuous, dark-colored, very persistant and adhesive, suggests a precipitation or coagulation phenomenon with the production of an irreversible hydrogel, and this in turn

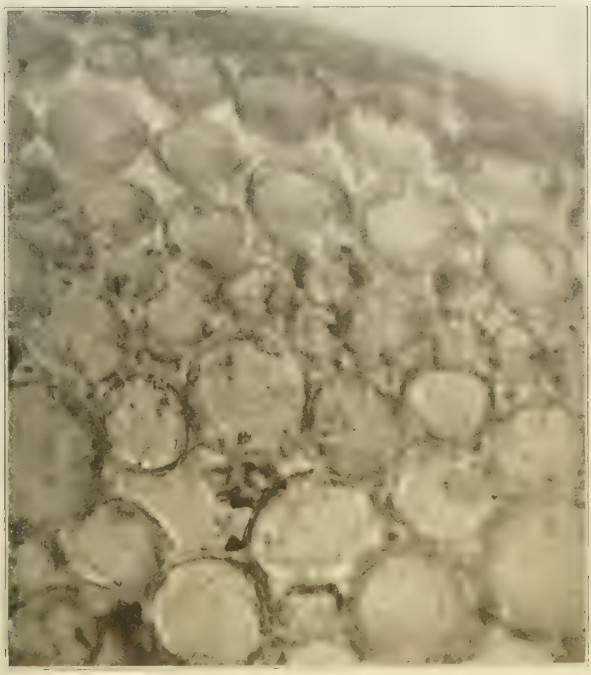

Fig. 28. Cross-section of healthy Bartlett pear petiole showing the cortical region. Magnified about 500 times. (Compare with Fig. 27.)

would mean the loss not only of water but of dissolved substances as well. It may, therefore, be assumed that most if not all the nitrogen requirements of this pathogen are met with material obtained from the protoplast. Although Tupper-Carey and Priestley *(39)* have shown that cell walls of meristematic tissues of broad beans contain complex proteins, there is very little known about such nitrogen compounds in cell walls of other plants.

The rapid destruction of cell walls and middle lamellas also suggests that these structures serve as a very excellent source of energy for this organism. This can be concluded not only from a study of these petal infections but also from studies of infected leaf blades, leaf petioles, flower receptacles, fruit, fruit stems, twigs, and limbs. The statement made by some investigators that cell walls are not affected by this organism can be questioned for the reason that one of the commonest histological symptoms of this disease is the production of cavities, frequently within the cortex, and sometimes within the phloem and xylem of all the plant parts that have just been enumerated, and these cavities are merely the outcome of wall destruction engendered by this invader. As has already been noted and as the photomicrographs clearly show, there is no evidence of a mechanical push or strain of bacteria on the walls. While there is a possibility that in other organs and tissues the action may be different from the one described, yet considering the delicacy of these petalary walls as compared with the much more substantial ones that exist in the cortex, phloem and xylem, of stems, fruits, petioles, etc., there does not appear to be much likelihood of such action. Of course it is conceivable that given different types of tissues which in terms of nutrition may mean a difference in kind and amount of nutrient materials, the metabolism may be expected to be different. Thus the enzymes liberated by the pathogen in unlike types of media may be quite dissimilar, as has been conclusively shown for various microorganisms *(51, 15)*, but this mainly involves differences in chemical rather than in physical behavior. It is for this reason that the writer is in agreement with Stewart *(35)* when the latter questions the applicability of his enzyme studies of *B. amylovorus* on nutrient media consisting of beef bouillon. Harter and Weimer *(15)*, for example, found that *Rhizopus tritici* produced cell wall dissolving enzymes on various vegetable media when it otherwise failed to do so on beef bouillon. The fact that these petals do not contain chloroplasts or amyloplasts would also suggest that the main source of carbon lies in the cell walls, since the amount of carbohydrate materials present within the protoplasts would not in these instances be very ample. Likewise, Burrill's idea that starch is readily utilized by this pathogen has apparently been disproved by several investigators *(35, 34)*.

The statement made above that cavities are to be found in this disease not only in the cortex but also in the phloem and xylem requires amplification. While this appears to be true, as will be shown later, there is also no doubt that by far the greatest amount of wall destruction occurs in the outer cortex of different organs, and there is even a possibility that the cavities sometimes noted in the phloem and xylem may be due to secondary invaders which have followed in the wake of *B. amylovorus*.

However this may be, there can be no question that in stems, petioles, fruits, etc., this macerating action involves principally parenchymatous cells of comparative soft walls and involves very little tissue possessing hardened cell walls. This would suggest that the organism metabolizes certain types or stages of wall formation very readily and others rather sparingly. The phenomenon here involved may possibly explain the resistance

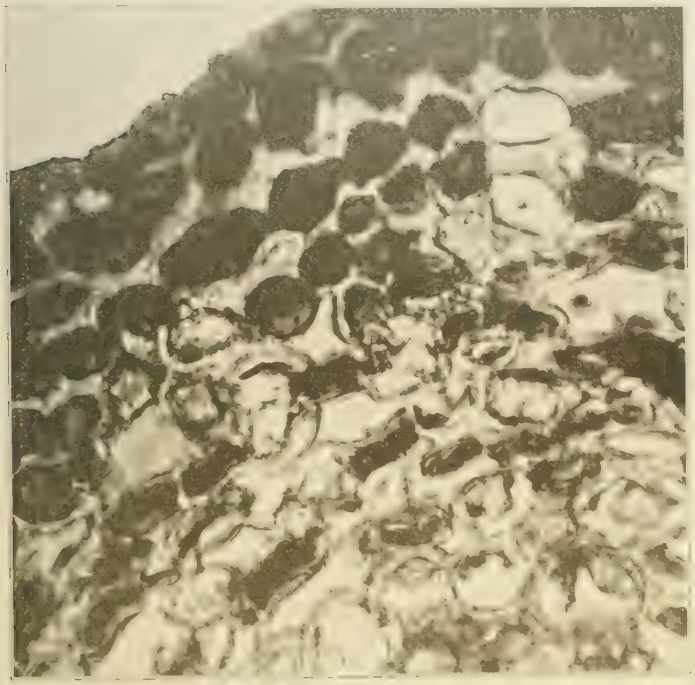

Fig. 29. Cross-section of a freshly infected Bartlett pear petiole showing bacteria within one intercellular cortical space (toward left). Bacteria in the other spaces lost by diffusion. Magnified about 500 times

to infection observed at certain periods in the growing season. It is very well known that blight usually assumes serious proportions in the early part of the growing season and is only of minor importance as the season advances. Numerous investigators have also commented on the fact that conditions which make for tender, succulent types of growth, such as enrichment of the soil by manure and other fertilizers, are conducive to

blighting, and with conditions arising that slow-up growth and induce hardening of the tissues, blight is likely to be far less serious. Can this be explained by the theory that has here been proposed, and, if so, how can it explain the occurrence of blight in older and supposedly non-succulent wood?

There is a marked tendency to look upon cell walls as dead, inert structures, which, when once formed, remain unchanged. The word cellulose being presented as descriptive of walls, is used as a sort of pigeon-hole in which all thoughts of wall activities are put out of sight. This is emphasized because so frequently the assumption is made that walls are passive structures which play a minor role in the economy of plant life. Now, whatever theory one adopts for the formation or deposition of cell walls, there is no question about the walls continuing to change by the addition of new layers or new materials, and it is even probable that as long as cells of high metabolic activities, characteristic of chlorophyllose cortical cells, remain alive and irritable, their walls are capable of being changed. This change may at times involve no more than addition or subtraction to the disperse phase of the colloidal complex of the wall, but this may be sufficient to render the older and supposedly fixed tissues more susceptible or resistant as the case may be. The imbibition of a sufficient amount of water by cell walls may be all that is necessary to start the rapid metabolic activities of a chance invasion by *B. amylovorus*.

This question impinges upon the method by which the fire blight pathogen initiates and extends infection. It has previously been recorded that in the petal infections under consideration the pathogen destroys cell walls very readily. This is to be observed in greatest abundance within the region around the original invaded area and is less common as one traces the infection toward the periphery of the diseased area where the invasion is most recent. In addition to this, there are also other signs of a slackening in pathological effects, particularly in the noticeable reduction in plasmolysis, irrespective of the fact that these peripheral cells are to be found ensconced by bacteria just as the older infected parts. Attention must be directed also to the fact that the infections here referred to had not yet run their full course and that the invaded area would have been further enlarged had the infections been permitted to develop fully. The only noticeable symptom observed in the peripheral cells is the production of a dark color, which very clearly delimits the diseased area. So noticeable is this correlation of discoloration with the immediate presence of bacteria that one can readily locate the marginal strands of bacteria by locating with low powered objectives the margin of the discolored area. In stained preparations the discoloration is likely to be unobserved because of the deposition of the staining material.

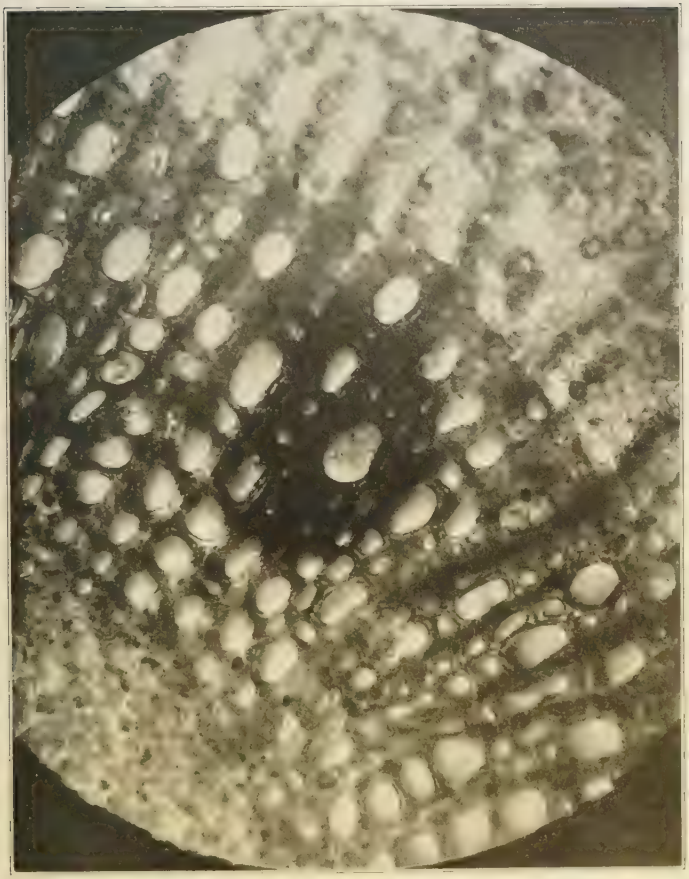

Fig. 30. Cross-section of a freshly infected Bartlett petiole showing bacteria within ducts. Magnified about 500 times. The discoloration of the xylem seen around the invaded duct is an artifact induced by over-exposing this region in the printing process. It was not present in the section.

What is the significance of this correlation? First, it is evidence of strict parasitism, and second, it indicates the manner in which the parasite accomplishes destruction. By the first is meant the absence of any evidence, with the exception to be noted later, that would indicate the presence of a diffusible substance capable of producing pathologic phenomena ahead

of the invader as occurs, for example, in sweet potatoes acted upon by *Rhizopus tritici*. The evidence indicates that the parasite attacks living cells and that pathogenic expressions are not produced until the invader has made its appearance. Not only is this evident in petal infections but also in all other types of infections which have been studied including those on leaf petioles, twigs, and fruit. It has already been recorded that the pathogen has been found within the xylem tubes without calling forth any disease symptoms either in the region around the invaded area or in tissues above, and when this is compared with other pathogens which are vascular inhabitants and which frequently produce wilting and other disease symptoms on leaves and stems that are considerably removed from the invaded area, the evidence is indeed substantial for the assumption that diffusible toxic products are not produced by *B. amylovorus*. The only possible exception that the writer has found consisted of a wall dissolution in cortical cells of leaf petioles in which bacteria were not observed in the affected walls. This, however, as will be discussed later, may have been due in part to faulty technic. In any case, even if it is assumed that wall dissolution occurs ahead of bacterial penetration, there is no evidence to indicate that any toxic phenomenon occurs within the living protoplasts of uninvaded tissues.

The second point mentioned involves the manner of invasion and disease expression. Once the invader has initiated infection it may of course be expected to advance as it grows and multiplies. The writer is inclined to look upon this advance or migration as a passive one engendered by the accretion of great numbers of bacteria rather than an active pseudopod-like movement postulated by Nixon. There is also no evidence that the bacteria are actively motile within the thickly populated strands. Having reached and surrounded a particular cell, the first noticeable effect is the production of a brown color which apparently stains protoplasts as well as walls, although the writer is not entirely convinced that the seeming wall discoloration is real or fancied. Similar reactions are known to be induced within the plant tissues by various means, one of the commonest ones being an internal oxidation phenomenon such as has been found in the black heart disease of the Irish potato (44). Does this suggest that the first effect is asphyxiation resulting from the exclusion of free oxygen by the surrounding bacteria? The slimy compact bacterial mass must interfere considerably with the gaseous exchange within the invaded parts, and, if this is true, how can these host cells avoid autolyzing their oxidative compounds? With such destructive exodation persisting there is little need of postulating the production of substances by the pathogen which are specifically toxic to its hosts, a theory which is sometimes offered to account for pathogenicity and which is

Fig. 31. Cross-section of a two-year old Yellow Transparent blight canker showing bacterial cavities both in cortex and in phloem. Bacteria lost in the process of sectioning. Material gathered near Fayetteville, March 13, 1927 and proved infectious. Magnified about 90 times.

well founded in some instances, particularly in pathogens which restrict their parasitism to only one or a few species of host plants or in which the pathogenic effects may be noted in regions removed from the actual seat of the parasite. But in this instance we are dealing with a parasite which is capable of entering through natural openings, which possesses an extremely wide host range, as Rosen and Groves (31) have previously pointed out, and which limits its activities to occupied territory.

If, therefore, one postulates the ability of this parasite to utilize for nutrient purposes certain compounds common to rosaceous plants, it is unnecessary to seek for specific toxins or poisonous ingredients which will account for its destructiveness. The thorough-going researches of Reichert and his associates

(28) on the specificity of various chemical compounds found in plants and animals, which makes it possible to determine relationships between individuals, points clearly to the fact that within one related group of living things one may expect certain chemicals which are more nearly alike than in unrelated groups. Thus the specific types of proteins, carbohydrates, etc., common to the Rosaceae may be expected to be different from those found in other families and this in turn may be seen as delimiting the pathogenicity of *B. amylovorus* by the availability or unavailability of these chemicals for nutritive purposes. Of course, this by no means explains all phases of resistance and susceptibility to the blight producer and our knowledge of resistant carriers suggests that in some instances the ability of a host to sustain growth of a pathogen is not correlated with disease expression. The theory here presented is partially comparable with the one developed by Dickson and his associates *(11)* for explaining resistance and susceptibility of wheat and Indian corn to *Gibberella saubinettii*. These investigators have found that resistance and susceptibility is correlated with the chemical composition of the cell walls and that inherited as well as induced immunity are both due to the same thing. Where this impinges on the theory here presented is that the kind of nutrients present in a given host forms the criterion of pathogenic activity, although in this case the activation of *B. amylovorus* and its resultant effects on host cells may be considered as involving considerably more than cell wall composition.

Recapitulating, the pathogenic behavior of the fire blight organism may be traced to its ability of metabolizing certain types and stages of cell wall formation and its complete surrounding of protoplasts which are thus deprived of free oxygen. This results in the death of the living parts of the cells, rendering them permeable to the passage of water and solutes, and making available the additional nutrients necessary for the growth of the organism.

While this theory is in harmony with all the observed facts detailed in these petal infections, there are so many gaps in our knowledge of parasitism in general that it can only be looked upon as a tentative suggestion. Thus, the entrance of *B. amylovorus* into a substomatal chamber of a young leaf having been accomplished, how does it acquire the initial push which permits it to occupy the surrounding intercellular air passages? The same question may be asked of any bacterial pathogen which invades through natural openings. Miss Haber's work *(14)* with *B. amylovorus* in wound inoculations of apple leaves, as well as Nixon's studies *(25)* on wounded apple sprouts, indicate that invasion of tissues following inoculation is very rapid, they having found bacterial strands which they designate as zoögloea within the tissues surrounding the wounds one hour,

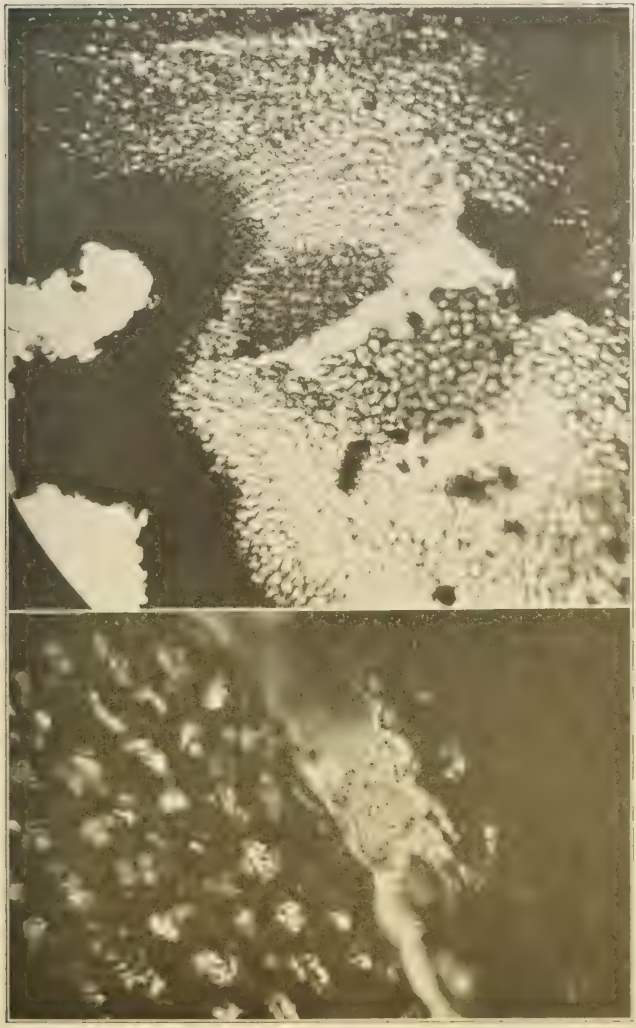

Fig. 32. Two-year old Spitzberg twig with cavities in the bark caused by *B. amylovorus*. Upper—magnified about 225 times; lower—about 450 times. A blighted shoot developed within the margin of this wintered canker. Material gathered April 20, 1927

and one and one-half hours, respectively, after inoculation. These being inoculations into wounds, it may be assumed that the invader finds nutrient materials available in the form of disrupted cells and may be looked upon as being offered a saprophytic existence. But in unwounded cells surrounding substomatal chambers, where do the nutrients come from? (In artificial spray inoculations these conceivably may be carried in the nutrient media of the pure cultures.) Of course, if Nixon's and Haber's idea of zoögloeal movement is correct, then all that would be necessary is to assume a bodily movement of the initial inoculum from the air chamber into the surrounding air passages. There is also another possibility.

It has been known since the early work of Dandeno *(10)* that leaves covered with water are capable of giving off organic and inorganic solutes. It is also well known that infections of various sorts, particularly those through natural openings, occur only in the presence of moisture and that in the absence of rains or heavy dews, infections are at a minimum. Assuming that the presence of water will call forth the excretion of solutes into the substomatal chambers, may not this aid in initiating a growth and reproduction of the invader sufficient to enable its penetration into the adjoining passages. This, coupled with the prevention of free gaseous exchange by the presence of the liquid plus bacteria within the air chamber, may be entirely sufficient to give the pathogen a start in its invasion.

Cytological and Histological Studies of Infected Pear Petioles

Unlike the infected pear petals which have just been described, it was found to be practically impossible to mount other organs *in toto* without obliterating interior views. This means that sectioning had to be resorted to with a consequent loss of most of the bacteria within the infected tissues, and this occurred in killed and fixed material as well as in living. Consequently the studies that are about to be reported on infected pear petioles must be interpreted with this loss in mind.

The material utilized consisted of fresh infections that had been obtained in the greenhouse by inoculating succulent pear shoots. The infections thus obtained would frequently run up into the subtending leaf petioles and midribs (see Fig. 16), thereby offering good material which could easily be sectioned. Miss Bachman *(3)* used fruit peduncles in part of her studies, as has already been mentioned, but she evidently confined these to killed, fixed, paraffin imbedded and stained material. Likewise, D. H. Jones *(21)* used fruit peduncles, which, morphologically, are quite comparable to leaf petioles. Immediately after severing diseased shoots plus leaves, the material was brought into the laboratory and sectioned at once. Held between pith,

Fig. 33. Cross-section of Winter Nelis pear canker on two-year old limb showing pockets of bacteria in the outer cortex and the formation of a corky layer separating diseased from healthy tissue. Material gathered March 8, 1928. Magnified about 500 times.

the leaf petioles could be easily sectioned with a razor and when mounted in water, and immediately examined under the microscope, the sections were comparatively free from artifacts which might be induced by the handling. As a check healthy petioles were similarly treated and compared with the diseased ones.

When a whole cross-section of an infected petiole is brought into view, Fig. 26, the diseased area is to be noted chiefly in the

cortical region existing between the epidermis and the tenth layer of cells. It will be recalled that the ventral surface of a pear petiole is flat or grooved while the dorsal one is rounded, and the peculiar thing noted was the frequency of infections extending along the margin of the groove where the tissues, with the exception of the extreme basal parts, extend above the regular contour and are richer in chlorophyll, the latted imparting a greener color to these ridges. From the infected cortical cells of the ridges, the diseased parts extend downward into the dorsal regions. While infections may be found involving more or less of the whole cortical circumference, they are frequently found sharply delimited by the ridges and not extending into the intervening flat or grooved area, as can been seen in Fig. 26. The reason for this is not at all evident. Under low magnifications the diseased area may be readily distinguished from the healthy parts by its extreme dark-brown or blackish appearance.

When this is examined under higher magnifications it is seen that the black color is due almost entirely to the appearance of the diseased protoplasts. The cell walls in contrast with the protoplasts show little discoloration. As in the petal infections which have already been described, there is considerable wall destruction resulting in this case in the frequent production of cavities or pockets within the cortical tissues. Whether this was due to chemical dissolution or to mechanical pressure could not be determined with certainty, owing to the loss of most of the bacteria occasioned by the manipulation. However, if weakened, thin places in the walls are best explained by enzyme action rather than by a physical push, then it may be said that here also the evidence points toward chemical dissolution, and in addition to this evidence, there can be seen a very marked swelling and lamellation of many wall parts within an infected area. This can easily be observed when a diseased and a healthy section are mounted alongside of each other (compare Fig. 27 with Fig. 28), otherwise it is likely to be overlooked. The separating out and the swelling of layers within a wall surely suggests a chemical phenomenon. It is frequently to be seen in parts of walls where the bacteria are not present. While this absence of bacteria may in part be due to the washing out of the bacteria from the sections, it does not explain the very evident lamellation observed in walls that still connect adjoining cells. From this viewpoint, one is almost forced to conclude that wall destruction is in part at least accomplished ahead of bacterial invasion. Microchemical tests which may help in a solution of this problem will be undertaken in the future. Unlike the young petals in which the intercellular spaces are small and rather infrequent, petioles show large, numerous air passages with the pathogen often found occupying the whole passage in a cross-section of infected regions (see Fig. 29). Crystals of

Fig. 34. Apple canker (variety Spitzenberg?) gathered March 10, 192-. cavities within outer cortex. This was a smooth-surfaced, indefinit margined canker without any cracks, but note the cork layers separatin diseased from healthy tissues. Magnified about 300 times.

various shapes and sizes are to be observed in occupied as well as in unoccupied air spaces. Diseased protoplasts very greatly resemble those in petals, showing in addition to the discoloration, very marked plasmolysis, hardening, and resistance to dissolution. The plastids are no longer observable, and, indeed, no other well defined bodies of any sort are to be detected as can be seen by comparing Fig. 27 of a diseased cortical region with Fig. 28 of a healthy one. In addition to the occupation of air passages the bacteria may also be frequently observed within the cell walls taking up the space left by the retreating, plasmolyzed protoplast (see Fig. 27), but in no case have bacteria been located with certainty within the latter. It is thus evident that almost all the cytological and histological phenomena involved in cortical invasions of leaf petioles are very comparable to those described for petal infections.

In addition to cortical invasion, the bacteria have also been found occupying ducts within the vascular bundles (see Fig. 30). As will be described more fully later, vascular invasion is far more common than has been recognized, although Miss Bachman called attention to this in her studies of infected shoots. Inasmuch as cortical infection of petioles has usually been found limited to the outer layers of cells and has only in a few instances been found in a continuous extension into the deeper xylem tissues, the question is, how did the bacteria obtain entrance into the xylem vessels? These petiole infections were extensions from diseased stems, as previously mentioned, and it will be shown later that within such stem infections the bacteria can be found within cortex, phloem and xylem. It may, therefore, be reasonably assumed that xylem invasion of petioles are much like the cortical ones, representing extensions in both instances from invaded stem tissues; but, while the whole stem may be more or less occupied, as the disease producer extends into the subtending petioles it becomes more localized so that widely separated invasions are to be noted. By far the most serious damage produced in these petioles is to the cortex, the vascular system evidently suffering very little even though the parasite be present. The death of the leaves which eventually accompanies the stem and petiole infections is very largely due to the death of the stem and rarely to the direct action of the pathogen on petioles or leaf blades. Proof for this may be seen in the fact that when bacteria are directly applied to susceptible leaves, resulting in infections which in number and extent compare with indirect infections, the leaves do not ordinarily die until the pathogen has taken a reverse passage from leaf to stem. When the latter thus becomes invaded, the leaves soon perish. Therefore the death of the leaves is in most instances the indirect expression of stem destruction.

Fig. 35. Canker of three-year old pear limb gathered March 18, 1927. Longitudinal (radial) section through margin of canker showing a layer of cork separating diseased from healthy tissue. Magnified about 500 times.

Cytological and Histological Studies of Infected Stems

The paucity and conflicting nature of the information concerning the pathological cytology and histology of diseased stems, in spite of the fact that these organs constitute one of the most common and often the most serious seat of the disease, has already been commented upon. For the studies which are about to be reported, diseased stems, young and old, of apple and pear gathered at different times of the year, from both artificial and natural infections, were sectioned with a sliding microtome, occasionally supplemented by paraffin imbedded, stained preparations. As a whole staining was found to be a hindrance rather than a help, inasmuch as practically all the stains in common use color these woody tissues very heavily and the bacteria but slightly. When the latter are sufficiently stained, then the tissues appear so dark that it is very difficult to discern details of structure and next to impossible to obtain satisfactory photomicrographs. When a triple stain is used, another factor intervenes which makes pathological details less observable and much more difficult to photograph. Different colors have of course different wave lengths, which under the microscope would make objects at one level appear at various levels of focus. Even with the use of apochromatic objectives, many of which are corrected for only two colors, there would still be no sharp focus for all the colors, and the use of color screens is only partly helpful. From this viewpoint, one color is better than three and when sections of woody tissues with walls of high refractive indexes are to be studied, there is no particular advantage to be gained by any staining. In most of the photographs here shown, while representing somewhat low magnifications, most of them being not more than 500 times, the bacteria are nevertheless clearly observable. It is obviously of considerable advantage to have as much tissue as possible under focus at one time and it was found that by using a four mm. achromatic objective with a number 10 ocular the unstained pathogen could be clearly distinguished within the tissues by taking advantage of oblique light. Such light materially increases the resolving power of a lens, and in viewing or in photographing a particular object obliqueness can be obtained by lowering and raising the condenser. With the help of different color filters, particularly brown and blue or sometimes with only a ground glass screen, the one to be used depending upon the color and opaqueness of the section and upon the quality of light, several exposures of one object can be made on the same plate, using perpendicular beams of light as well as oblique. In this way objects possessing diameters of one-fifty thousandths of an inch can easily be resolved with lenses possessing rather low numerical apertures. It was also found that for observing

Fig. 36. Cross-section of cankered apple twig [illegible] of bacteria close to the phloem. [illegible]

the finer structures, water makes a far superior mounting medium to Canada balsam. This can be explained by the fact that Canada balsam, having a higher refractive index than water, would tend to level the markedly different indexes of protoplasm, cell walls and air spaces.

One of the commonest seats and frequently the only one, is the outer cortex, as various investigators have previously reported. But, in addition to this, the disease producer can readily be found throughout the whole range of tissues, with the excep-

tion of the epidermal layer. In studying sections of diseased stems, the most impressive thing is the destruction of the outer cortex, resulting in large and small cavities (see Figs. 31, 32, 33). Nevertheless, by far the most serious effects on the infected twig or limb result not from cortical destruction but from invasion of the phloem and cambium. It is the destruction of the latter and not the cortex which results in the death of the twig or limb, and when the invasion encompasses the whole or large part of the circumference, then the parts above the infected region perish sooner or later. It is in this manner that the death of twigs and limbs occurs. From a reading of the literature, it is evident that some investigators, as for example Waite, clearly understood this but others do not seem to have done so, or if they did, said little or nothing about phloem, cambium and xylem invasion while emphasizing cortical ones. This perhaps is due to the fact that the cortex is much more easily sectioned than the inner bark and xylem, and it is very difficult to avoid tearing the cambium in such woody material. Of the photomicropraphs of diseased stems which are here presented, diseased cambium views are conspicuous by their absence for the reason that sections showing cambium destruction were usually so badly mangled in the process of cutting that no satisfactory photographs could be taken. It is relatively easy to obtain good sections of healthy cambium (see Figs. 42, 46) in tissues adjoining diseased ones, but within areas in which the invader has reached the cambium, the destruction is usually so complete that upon sectioning, the bark almost invariably separates from the wood. As a consequence, cambial disturbance is likely to be overlooked. While this may be properly emphasized, there is no question that the invader often goes no farther than the outer cortex. When this occurs, the disease appears as a localized infection often in the form of a canker, which does not seriously interfere with the tissues which may happen to be above the infected area. Field evidence, while not conclusive, seems to indicate that in such infections the bacteria are likely to die out readily, with the host sooner or later reacting by laying down a corky layer which walls off the diseased from the healthy tissues.

These suberized layers may or may not be evident on the surface. When they do appear there, separating diseased from healthy tissues, they are usually accompanied by a marginal crack or rift, which together with the suberization produce a well defined margin. This may be in the form of a clear cut, regular line completely encircling the circumference, or it may take the form of an irregular, jagged line running in part longitudinally. Suberization occurs not only in those infections which are strictly confined to the cortex but also may frequently be found in diseased twigs and limbs in which the disease pro-

ducer has invaded the phloem, cambium and xylem and has killed both the outer and inner bark. It will be shown later that, while this corky layer has walled off the invader in the cortex preventing its spread in a vertical direction, it has not materially influenced the passage of the bacteria in the inner tissues.

When a corky layer is not observable at the surface, this does not mean that a canker of an indefinite margin type still

Fig. 37. Cross-section of an artificial infection on Bartlett pear shoot showing bacteria in one duct and in an adjoining medullary ray cell (near center). Material sectioned within 48 hours after inoculation. Magnified about 500 times.

capable of spreading has been produced. Indeed, one of the commonest reactions to be found in histological sections of diseased stems is the production of suberized layers, separating diseased from healthy cortex (see Figs. 33, 34, 35) when the surface of such cankers show no indication of a well-defined, suberized margin. The writer has found no evidence in the literature that this has been previously recognized. The special significance that may be attached to the finding of corky layers

within cankers that superficially appear to have remained unchecked, rests upon the fact that outward signs of diseased areas cannot properly be relied upon as criteria for distinguishing hold-over cankers from non hold-over ones. Not only is this true for cases in which the canker margin appears indefinite but also for the reverse where, according to the definite, cracked, corky-margined exterior of cankers, one would expect the pathogen to have died out but in which the disease producer has been found to be alive and infectious, as can be seen by an inspection of Table 2. The table shows that about 50 per cent of the cankers which were found to have harbored the germ during the winter and early spring were of the definite, cracked-margin type.

With reference to the finer histological and cytological changes occurring in cortical stem lesions, the effects parallel closely the ones described for cortical invasions of petioles with but slight differences. Here also the pathogen is found working its way along air passages as well as between walls, which it destroys with as much avidity as in petiole cortex or in petal mesophyll. The protoplasts show the same discoloration, plasmolysis and rigidity, and the parasite can again be found within walls surrounding the dead protoplasm. There is, however, one marked difference between cortical invasions of petioles and that in stems, namely, the relative localized nature of the former, as has previously been described, and the frequent diffuseness of the latter. In stems the parasite does not remain confined to the outer cortex but frequently travels radially into the interior, making its way past the bast fibers and attacking inner phloem, cambium, xylem and pith. Owing to the loss of bacteria in the process of sectioning and mounting these woody stems, the writer has not been able to follow the path of the pathogen as clearly as in the petal infections. Nevertheless, bacterial pockets and cavities have frequently been observed close to the phloem (Fig. 36) and in the inner phloem (see Fig. 31) as well as in the region of the cambium, and the indications are that here also the invader has destroyed cell walls by chemical dissilution rather than by mechanical pressure. In this connection it is to be noted that a disturbing factor frequently intervenes which makes it extremely difficult to determine the histological changes which may be ascribed to *B. amylovorus* and those brought about by secondary invaders. It has frequently happened that bacteria found within tissues showing pockets or cavities, which in form or size are indistinguishable from *B. amylovorus*, are non-infectious and yield upon culture plates bacterial colonies of various sorts, many of which possess a yellow color. The extraordinary rapidity with which blighted tissues are invaded by secondary organisms has been noted in both artificial and natural infections, and one of these common

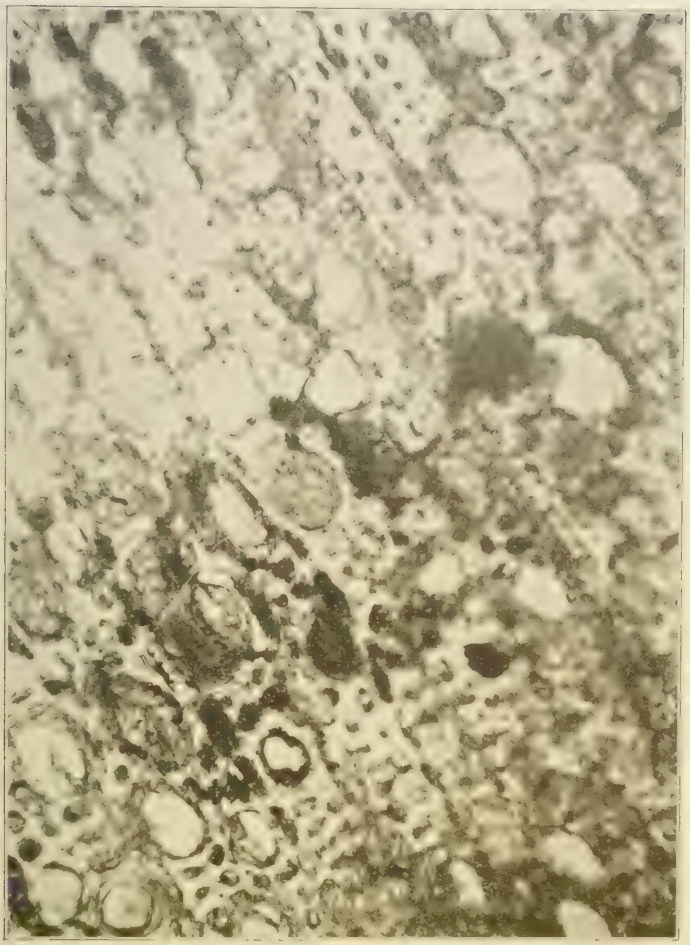

Fig. 38. Cross-section of three-year old Jonathan apple limb, taken from about one mm. below the margin of a blight canker showing granular (bacteria?) matter in a group of ducts. This group formed a dark-brown streak within the wood and was traced for a distance of about six inches, reaching into the four-year old portion of the limb. Magnified about 480 times.

Fig. 39. Upper—newly blighted spur at base of twig which had blighted the year before on Spitzenberg apple. Gathered April 18, 1928. Lower—Cross-section of twig shown above taken at level of wood running into the newly blighted tissue, showing bacteria within a xylem tube. Are the bacteria migrating from the new to the old infection or vice versa? Magnified about 500 times.

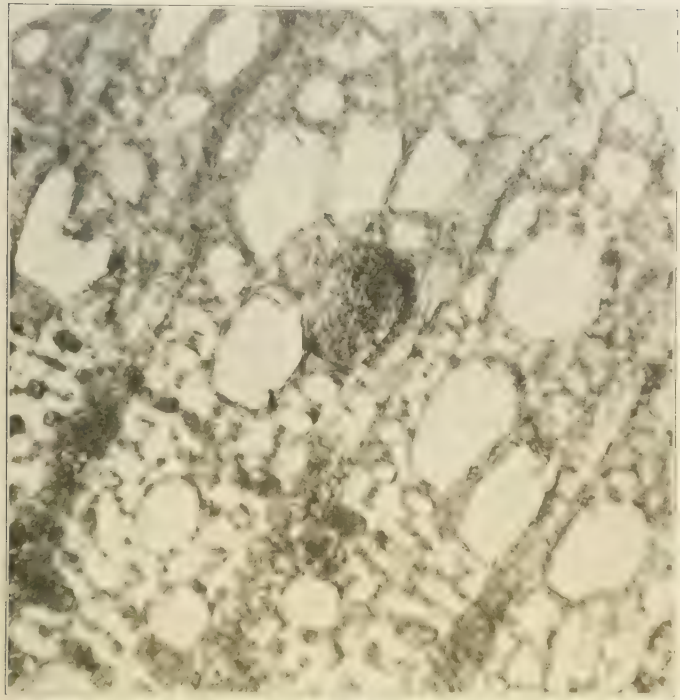

Fig. 40. Cross-section of a healthy Kieffer pear shoot gathered April 24, 1928, showing bacteria within a duct of the current spring wood. The shoot arose from within the margin of a body canker which showed no signs of activity in the way of expanding margin or of oozing. A similar shoot, also within the margin of this canker was macerated, used for inoculation experiments and yielded typical infections. Magnified about 500 times.

invaders has been made the subject of special studies which will be reported elsewhere. While these secondary invaders have created a difficult problem in determining the exact changes which have been brought about by *B. amylovorus*, there can be little question that the latter is primarily responsible for the destruction of inner bark tissues and that it frequently gains entrance into the xylem by virtue of its power to invade and destroy these tissues.

It is truly remarkable what little attention has been paid to xylem invasion in spite of the fact that the able pioneer investigator of this disease, Burrill, makes mention of it and it has been noted by others as well. The fact is that one of the com-

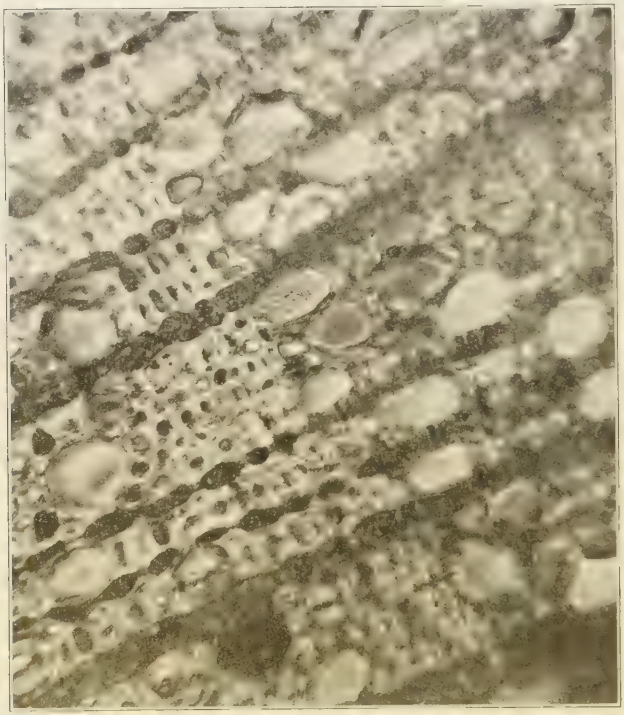

Fig. 41. Cross-section of two-year old Jonathan canker, taken about one mm. below margin of the canker, the latter being smooth and without cracks. Note bacteria within a duct close to the center adjoining several other ducts filled with a gummy non-granular material as well as a granular substance, resembling bacterial bodies. Attempts at isolating *B. amylovorus* from this canker failed. Material gathered Feb. 23, 1927. Magnified about 500 times.

monest internal symptoms of this disease is the marked discoloration of the xylem (Fig. 48) in the infected regions in contrast to various other canker-producing apple diseases such as blister canker, bitter rot canker, black rot canker, and blotch canker, prevalent in this state, and in which no such discoloration of woody elements are involved. In what manner the xylem is reached by the pathogen has not been definitely established. Burrill conceived this to have occurred by means of the medullary rays and one or two other investigators have concurred. But this is surely not the case in all instances for the reason that an invaded ray reaching from phloem to xylem is extremely rare. It is true that one or more invaded ray cells may occasion-

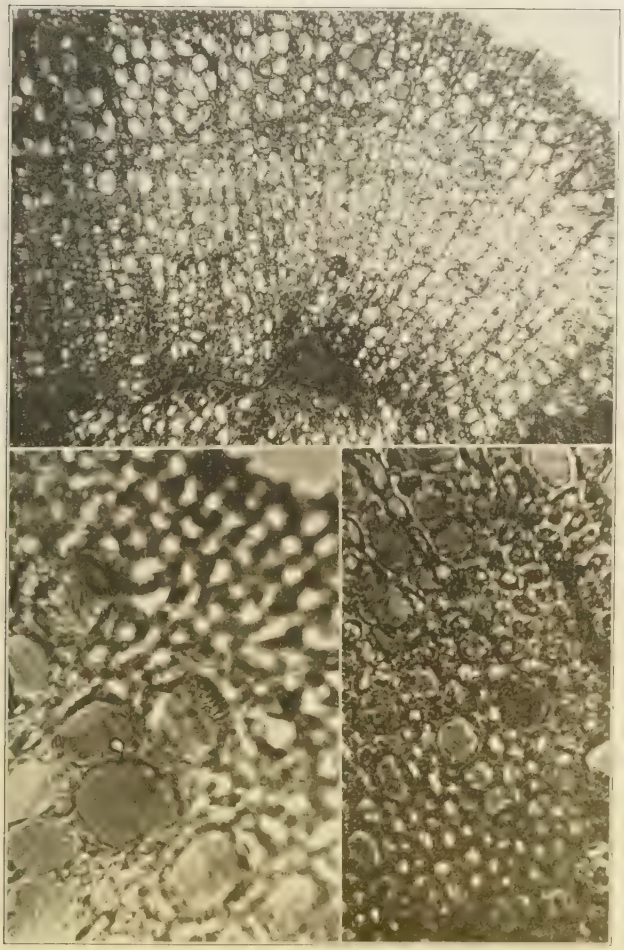

Fig. 42. Cross-sections of three-year old Jonathan twig taken several inches below margin of a swollen, irregular canker. Blackish streaks were found running in the wood for about six inches below margin of canker. Upper—gross view from cambium to pith, magnified about 220 times; lower left—gummy, granular material, more or less resistant to cutting within ducts of this season's growth, resembling bacteria; lower right three-year old wood near pith, bacteria in duct near center; lower figures magnified about 440 times

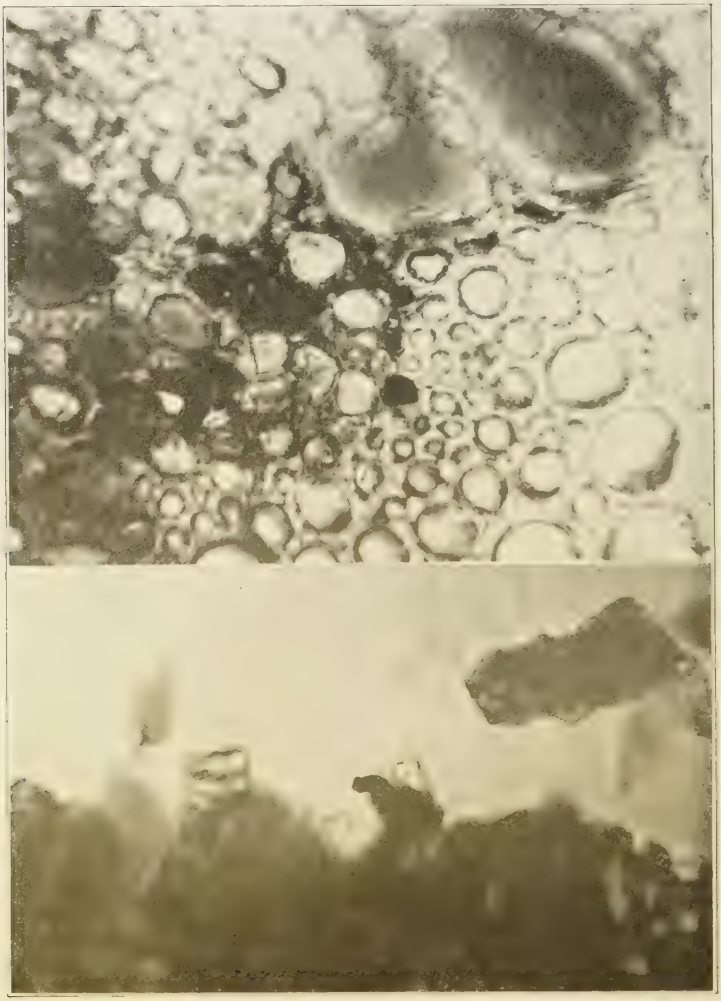

Fig. 43. Upper—cross-section of Winter Nelis pear canker showing gummy matter within cavities consisting of disrupted ducts; lower—gummy matter squeezed out of cavities, mounted in water. (Note comparative insolubility of the gum as shown by the clumps free from cell walls, also individual bacteria in upper part separating out from the ducts within the xylem.) Magnified about 500 times.

ally be seen (see Fig. 37) alongside of a xylem duct, but this may just as well be explained as an invasion from the duct to the ray as vice versa. It of course may be argued that invasion from bast to xylem need not necessarily occur in one row of ray cells but may be envisioned as one of irregular jumps, so that the final infected ray cell in juxtaposition to a xylem tube is at an entirely different level from the cell invaded in the region of the phloem, comparable to a checkerboard arrangement. The writer, however, has seen no evidence for this but has observed other phenomena which tend to question it.

When a section of discolored wood is examined, it is seen

Fig. 44. Bacteria in a water mount squeezed out from the ducts of a blighted Jonathan apple twig, by applying pressure to the cover slip. Artificial inoculations showed these to be non-infectious. Material gathered Feb. 15, 1927. Magnified about 650 times.

that the discoloration is essentially due to the intense coloring within the ducts plus adjoining woody fibers (see Fig. 38). Nearby ray cells may or may not show such discoloration (Figs. 39, 40, 41). Very frequently bacteria can be clearly recognized within the ducts, as may be noted in these photomicrographs, but at other times the ducts are filled with a granular matter, suggesting at times decomposing bacteria and at other times merely a granular gum (see Fig. 42). This latter material is with difficulty dissolved in water (Fig 43) when other ducts

filled with true bacteria readily separate out in a water mount (see Fig. 44). Out of many attempts to prove that such bacteria are alive and capable of producing infection, only one was successful. This is the one that has previously been recorded as consisting of a healthy pear shoot, the base of which was attached to a body canker. As already noted, the shoot showed no evidence whatever of outward signs of disease, and, when histological sections were made, bacteria were noted only within a few xylem ducts, none whatever appearing in outer or inner bark. When comparable tissues of another healthy-appearing shoot existing close to the one used for histological purposes and also found within the margin of the same body canker was surface disinfected, macerated in water and inoculated into succulent pear shoots, typical blight was produced. As no signs whatever of any bacteria were to be found in the bark, in this shoot, the evidence appears to be ample proof for the passage of *B. amylovorus* within the water ducts of the current spring growth.

If the occupied ducts only rarely contain viable and infectious masses of *B. amylovorus*, then what is the constitution of these dark clumps? If one compares a clump of *B. amylovorus* obtained from a pure culture of the organism grown in nutrient broth, (Fig. 45), the similarity of appearance of such clumps to those found within some xylem elements is indeed striking, but when the latter are carefully examined it is noted that even at the same cross-section level there is a marked difference in the occupying masses of the different ducts. Thus an inspection of Figs. 42 and 43 will show that within one level there are ducts filled with comparatively good sized particles, very similar in appearance to a clump of *B. amylovorus*, others with much smaller particles, suggesting partly or wholly decomposed bacteria, and still others with a finely granular material much resembling a gummy non-living mass, such as F. R. Jones *(23)* has shown in diseased ducts of alfalfa plants. Cavities within the xylem, although not as frequent as in the cortex or phloem, are not uncommon (Figs. 43 and 46) and are often found occupied by a granular material which suggests either decomposed bacteria or gum. These cavities strongly suggest Nixon's "lysigenous" structures and the type of mater-

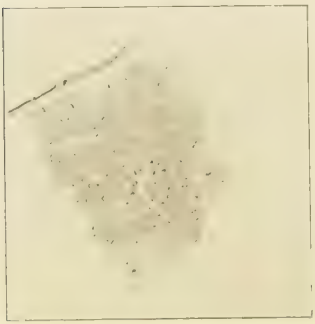

Fig. 45. Clumping of *B. amylovorus* in a pure, nutrient broth culture, six days old. Magnified about 500 times.

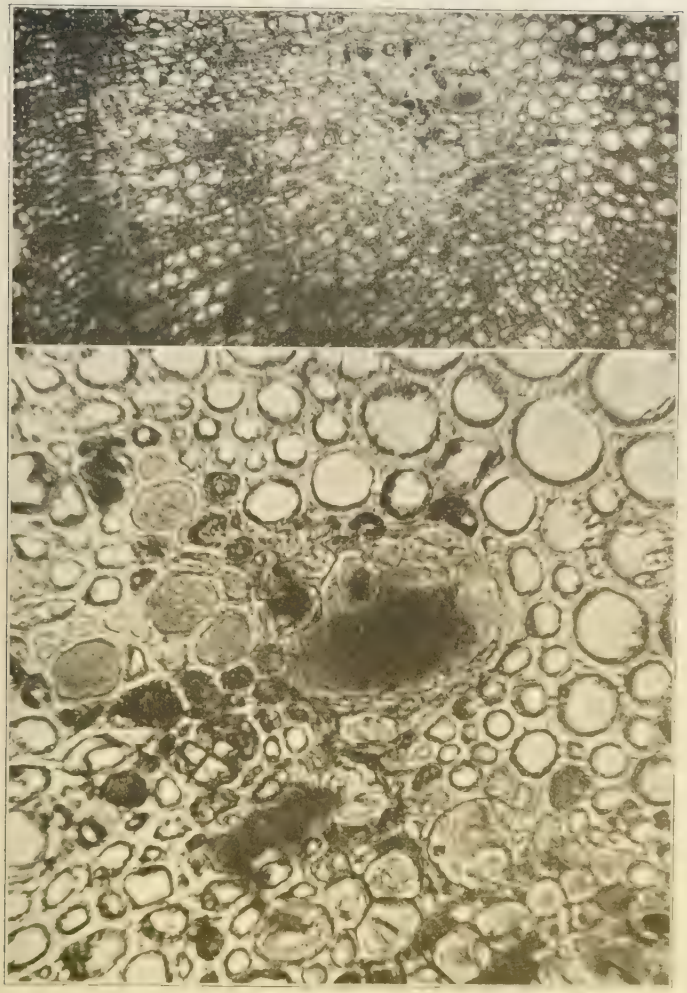

Fig. 46. Cross-sections of Winter Nelis pear canker showing gum and bacteria-like bodies within the wood close to the pith. Note the fair sized cavities partly filled with this gummy material. Upper—gross view from cortex (left) to pith (right), showing relative position of the gum within ducts near pith, magnified about 250 times; lower—enlarged view of ducts near the pith showing gummy bacterial material within individual ducts as well as in a pocket (lysigenous cavity?), magnified about 500 times. (See Fig. 43.)

ial within them as well as that in adjoining cortical and phloem regions bear marked resemblance to Nixon's "cysts". Rounded up, granular masses have also been observed in the pith (Fig. 47), but about 50 different attempts to produce infection with material containing such structures, were negative.

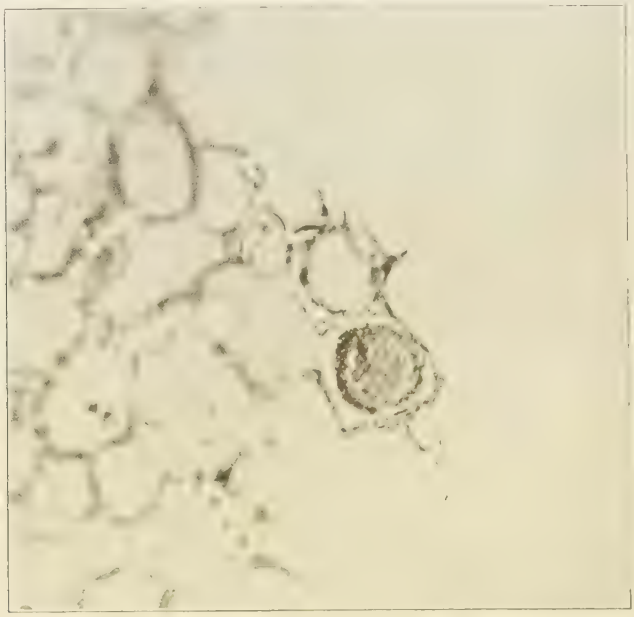

Fig. 47. Cross-section of blighted Jonathan apple twig gathered March 7, 1929, showing granular, rounded up mass of material within pith cell, resembling Nixon's "cyst" formation. Magnified about 500 times.

In concluding the description of this histological work on stems, attention is called to the fact that, while the pathogen has been found capable of invading all the tissues from cortex to pith, the studies have not clearly revealed that any one tissue or region is primarily responsible for carrying the disease producer overwinter. Specific evidence has been presented showing that it can be found in xylem ducts in the early spring, but this does not exclude the possibility that other tissues may likewise be involved. While the writer has spent considerable time in attempting in blighted material to separate outer and inner bark as well as xylem, and in making inoculations with macerated material from the different tissues, obtaining an apparent

differential effect, he is not prepared to accept the results. The reason for this is that even with the utmost care in handling, including the flaming of the knife before every cut, there is no assurance that the bacteria from one tissue have not been carried by the knife to other tissues in the process of cutting. However, from what has been said, it is very evident that the pathogen may at times live over winter in deeply imbedded tissues and would therefore be difficult if not impossible to reach with any surface disinfectant, or surface scarification. While such treatments may be expected to be effective in those cases where the pathogen is imbedded in more or less superficial tissues, they are not likely to be of value in the case of deep seated invasions.

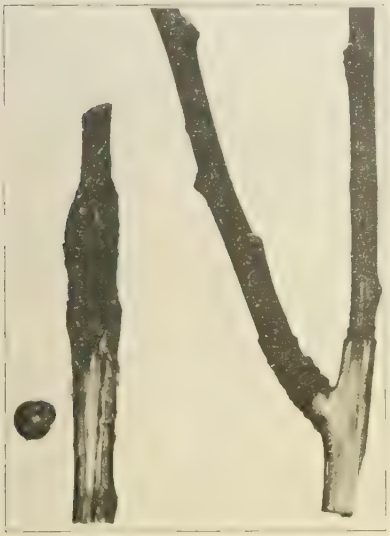

Fig. 48. Three-year old Jonathan limbs with blight cankers, gathered Feb. 12, 1927. Canker at left with black streak within xylem extending for several inches below outward signs of the disease; canker at right with dark discolored wood for several mm. below margin but with no elongated black streaks. Masses of bacteria were observed in ducts composing the streak seen on the left.

SUMMARY AND CONCLUSION

The common belief among Arkansas apple growers that fire blight of apples is due to blighted pear trees carrying the parasite over winter has made it desirable to undertake an investigation to determine the facts in the case and also to find a more adequate control of fire blight on apples than exists at present.

A review of the previous literature of this subject has shown that very few investigators have claimed that pears rather than apples are responsible for carrying the blight producer over winter. As a basis for an adequate study of the problem, the following specific lines of investigation were undertaken: First, the range of hosts of the fire blight pathogen in Arkansas; sec-

ond, general field studies of resistance and susceptibility, and overwintering on apple and pear; third, observations on early spring oozing of apple and pear cankers; fourth, attempts at isolating the parasite from overwintered apple and pear cankers; and fifth, cytological and histological investigations of (a) floral infections, (b) petiole infections, and (c) stem infections.

The study on host range has resulted in the discovery of several new hosts including the Burbank plum, cultivated rose, Vanhoutte spirea, Japanese or flowering quince, a species of Amelanchier cultivated for its edible fruit, and an oriental species of Crataegus. The latter two are here reported for the first time.

It is shown that aside from certain susceptible varieties of apples and pears, other hosts including quince are only slightly affected in Arkansas.

Concerning the field studies of resistance and susceptibility of apple and pear, it is noted that in absence of bloom, or when frost or other adverse condition injures the bloom, blight is not likely to be serious on either host.

Pear varieties are in general found to be more severely blighted than the susceptible apple varieties commonly grown in this state. Aside from inherent differences in susceptibility, this is partly due to the fact that pears being mostly grown for home use are frequently neglected, whereas apples constituting an important commercial crop are more carefully pruned and otherwise well taken care of.

In attempting to determine the possible relationship of early pear infections to subsequent epidemics of apple blight, daily observations from February on through the growing period, for three successive years, were made of the following: flowering periods of various pear and apple varieties, weather conditions especially at blooming time, observations on the date of first signs of blight and its possible relationship to any nearby infectious material, and, finally, the severity of blight throughout the season on different hosts. Coupled with these were field studies of early spring oozing and laboratory investigations of blighted apple and pear material.

It was found that pears bloom one to three weeks earlier than most apple varieties and that, unless something happens to the pear bloom, the blight may be expected to appear first on pears. In other words, if bloom blight develops, it must necessarily appear first on pears rather than on apples because of their earliness of blooming. It is obvious, therefore, that unless this is taken into consideration one would be inclined to draw the erroneous conclusion that pears are responsible for carrying the disease producer over winter because the disease is frequently noted first on pears.

All the field and laboratory studies clearly indicate that the parasite is frequently carried over from season to season on apples as well as on pears. Thus in 1927, when there was very little pear blight owing to the almost total destruction of pear blossoms by early spring frost, apple blight was very severe on susceptible varieties. Conversely, in 1928 and 1929, when pear blight was very common, apple blight was only moderately abundant.

In 1927 blight was first noted on apples, while in the two succeeding years it appeared on pears first and was noted six days later on apples in each year. As artificial infection experiments conducted out-of-doors in the early spring season show an incubation period of 10 to 14 days, it may be questioned whether sufficient time had elapsed for the first infections to have developed on apples from the inoculum engendered by the earlier blight on pears.

Apple blight has been found to be very severe in orchards that were more than a mile and a half away from any pear trees, and, likewise, in other apple orchards of susceptible varieties located in the immediate presence of pear trees, blight was noted to be negligible. In other words, blight may or may not be prevalent on apples, irrespective of the presence of pear trees.

When severely blighted pear trees are present near susceptible apples, the disease is likely to be more common and severe than on apples not in the immediate vicinity of such pears. Likewise, in the presence of neglected apple trees, blight is likely to be more prevalent on well kept trees of apples or pears. As a whole, however, it would be to the interest of apple growers to remove all pear trees because they are so frequently neglected. Also, pear trees, developing a greater amount of blight in a given length of time than the susceptible apple varieties grown in this state, may be expected to produce greater quantities of infectious material. Owing to the relative scarcity of pear trees, their removal can be more easily enforced than any removal of neglected apple trees.

In spite of the fact that early oozing of overwintered cankers acting as the source of inoculum for initial spring infections has been almost universally accepted as an established fact, a thorough review of the literature shows that up to the present no adequate proof has been presented for this assumption. While such oozing may yet be found to occur early enough to account for the first infections in some sections of the country, a very intensive study of blighted pears and apples over a three-year period has failed to reveal any such oozing in the field in Arkansas. Nevertheless, laboratory investigations of overwintered cankers and blighted twigs of apple and pear, involving artificial infection experiments, have shown that the blight producer lives over winter in about two per cent of the

cases. It is shown that small twigs and limbs of apple and pear can carry the bacteria from one season to another, and that the importance of large limb and body cankers acting as sources of overwintering has been exaggerated in the past.

The finding of the parasite in wood blighted the previous year is not sufficient proof that such wood is responsible for the initiation of blight, but, coupled with field observations in which new blight is frequently found joining that of the previous year and in which cone-shaped areas of infection are to be found on apple and pear trees centering around such overwintered blight, the evidence indicates more clearly that the blight is in part initiated by blighted wood of the previous year. More work is necessary to answer this question satisfactorily.

In order to obtain freshly infected material for cytological and histological studies and also to determine the possibility of water-borne inoculum playing a role in early spring infections, artificial infections of floral organs and of very young apple and pear leaves were attempted by applying the parasite as a spray in the form of a water suspension. Infections were thus obtained in great numbers on very young pear and apple leaves and on floral organs showing, contrary to prevailing opinion, that it is unnecessary to postulate insect visitation as a means of producing infections either on leaves and twigs or on flowers.

Floral infections by means of application of water suspensions of blight producing bacteria were obtained on peduncles, walls of receptacles, calyx lobes and on petals. With the possible exception of petal infections, those on all the other floral organs as well as on leaves were induced by the bacteria gaining entrance through the natural openings, particularly through the stomata. The experimental evidence would, therefore, question the common belief that, aside from nectarial penetration, the bacteria can only gain entrance through wounds.

Petal infections on pears are described and it is shown that, while some of these resulted from invasions through wounds others could not be so relegated. Theories of bacterial penetration are discussed and an explanation attempted for bacterial penetration in the absence of wounds or natural openings.

Cytological and histological studies of petal infections, fully illustrated, are presented. The bacteria, following penetration, are found making their way between cells in the absence of air passages by their very marked ability to dissolve cell walls and middle lamellas. Protoplasts completely surrounded by bacteria are described, and it is noted that intercellular invasion results in marked discoloration, plasmolysis and change in physical state of the protoplasm. Wall materials are considered to serve as an excellent source of energy for *B. amylovorus*, while water plus solutes are postulated as being obtained from the protoplasts. Evidence is presented showing that this pathogen acts

within the tissues as a strict parasite whose destructiveness is brought about by its attack on materials present within certain walls and by its rapid encircling of protoplasts, eventuating in their asphyxiation and final death.

Infected pear petioles have likewise been subjected to cytological and histological studies, and the observations closely parallel those of infected petals. In these plant organs the cell walls of cortical tissues are noted as undergoing a distinct swelling and lamellation, indicating the production of cell-wall destroying enzymes acting in part in advance of bacterial invasion. In addition to cortical invasion, bacteria are also noted as being present within xylem ducts of infected petioles.

Similar studies of infected stems of pear and apple, gathered at different times of the year and involving both succulent and woody material, are described. Attention is directed to the fact that, while cortical invasions are most common in stem infections, nevertheless, the most serious invasions result in the destruction of phloem and cambium, and it is owing to the death of the latter tissues that plant parts above an infected region are killed.

The production of suberized layers in cortical tissues, separating diseased from healthy areas is described, and it is shown that such cork formation may be present within the tissues without any outward indication of the cessation of cortical invasion. It is also shown, on the other hand, that cankers possessing well defined, suberized and cracked margins are fully capable of carrying the parasite over winter. It is thus evident that the outward appearances of cankers cannot be relied upon in distinguishing hold-over cankers from non hold-over ones.

With reference to the cytological details of cortical stem infections, the same effects are noted as in petiole infections of cortex with the exception that, while the latter are more or less localized, the former are frequently diffuse, resulting finally in invasion of inner cortex, phloem, cambium, xylem and pith. Secondary invaders frequently follow *B. amylovorus* and make it difficult to determine the exact changes brought about by the primary invader and also make it difficult to distinguish the parasite from non-pathogenic, secondary organisms.

The very common occurrence of xylem invasion is described and figured and it is shown that ducts are frequently found occupied by masses of bacteria. The discoloration of the xylem within an infected region is due mainly to the color imported to the ducts and to the accompanying woody fibers.

At one cross-section level of both old and young stems, there is great variation in the composition of the masses of material occupying the ducts. In some cases normal appearing masses of *B. amylovorus* are evident, in others the masses consist of disintegrated bacteria, while in still other cases the

masses are composed of a finely granular, gummy material. The same sort of variation has been observed in other stem tissues.

These studies clearly show that surface disinfection or scarification of cankers cannot be expected to control the disease adequately because the parasite is so often deep seated within the tissues.

In conclusion, it may be said that as these studies show the adequacy of apple tissues as well as pear in carrying the blight producer from season to season, the destruction of pear trees will not result in the freedom from blight of susceptible apple varieties, and in so far as the emphasis is placed on the overwintering of the parasite on pear trees only and the eradication of the disease by the removal of pears, there is danger of accentuating the disease on the apple by neglecting the latter host.

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